QUALITY ASSURANCE MANUAL

For

INDIANA WASTEWATER LABORATORIES

1st Edition November 2002
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Acknowledgements

It is our sincere hope that this manual will help any Indiana wastewater facility that utilizes it, develop a laboratory Quality Assurance Plan that will, in turn, generate sound, scientific laboratory data.

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Our mission, as a group, is to be a useful resource to all wastewater facilities throughout Indiana. We hope that this document is easy to follow and helpful.

The IWEA Lab Committee
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PREFACE

This manual is being distributed as a guidance document for laboratory personnel. The manual contains quality assurance and quality control information, detailed methods for the basic parameters that are reportable with a National Pollutant Discharge Elimination System (NPDES) permit, checklists, and sample bench sheets. This manual should be considered a tool that a wastewater laboratory can utilize to generate quality data.

The principal parameters monitored and reported for municipal permits include Total Suspended Solids, pH, Biochemical Oxygen Demand and may also include Total Residual Chlorine, and/or Nitrogen as Ammonia, and/or Total Phosphorus. Other municipal permit parameters will include, but are not limited to, *Escherichia coli* (*E.coli*), certain metals and oil and grease.

It is an old axiom that the result of any test procedure can be no better than the sample on which is it performed. Obtaining good results will depend to a great extent upon five major activities:

1. Collecting representative samples
2. Proper sample handling and preservation
3. Adhering to adequate chain-of-custody and sample identification
4. Adequate quality assurance and quality control
5. Properly analyzing the sample

These areas are equally important for insuring the NPDES reported data is of the highest validity and quality.

Monitoring and reporting effluent discharges under a (NPDES) permit requires specific test methods. These approved method numbers can be found in the latest edition of the CODE OF FEDERAL REGULATIONS, PROTECTION OF THE ENVIRONMENT, 40, Part 136. Only these methods are allowed for reporting purposes on the Discharge Monitoring Report (DMR) and the Monthly Report of Operations (MRO). Not every approved method is contained in this manual. The methods identified by number can be found in either: *Methods for Chemical Analysis of Water and Wastes, EPA-600/4-79-020,* or *Standard Methods for the Examination of Water and Wastewater, 18th Edition, 19th or 20th Editions.* **(NOTE: The 18th, 19th, and 20th editions of *Standard Methods* are now approved and all approved methods are listed in the new version of 40 CFR 136 dated 10/23/02.)** One or both of these references or copies of the methods should be a part of every wastewater laboratory.

Certain test methods may be specified for certain parameters in the NPDES permit. The methods specified should be capable of detecting that parameter at the limits imposed in the permit. If a method is not specified and doubt arises as to the acceptability of the method, call IDEM's Office of Water Quality, Operator Assistance Section.

QUALITY ASSURANCE and QUALITY CONTROL

I. Introduction

Environmental data produced in your laboratory is an estimate of the true values for the parameters (pH, Total Suspended Solids, Biochemical Oxygen Demand, etc.) being measured. The results you obtain are influenced by natural changes in the samples plus errors that occur during the collection and analysis of the samples. We realize, that as laboratory personnel, we have little if any control over the natural changes that occur in the samples. But we can reduce and potentially eliminate those errors that result from the human factor involved with the sample collection and analysis procedures. The focus of this section of the Quality Assurance/Quality Control document will be to provide you with the information necessary to help reduce those errors.

Let's begin by defining some terms relating to Quality Assurance and Quality Control:

Quality Control - the daily functions carried out during the collection and analysis of samples to help produce accurate and precise results;

> Examples: Proper cleaning of sampling equipment and bottles, calibration of testing instruments, analyzing blanks, replicates, and spikes

Quality Assurance - the development of the procedures used during sampling and analysis needed to produce accurate and precise results, plus the review of the results to determine if the developed procedures are adequate;

> Examples: Training of personnel regarding proper sampling and analysis techniques, reviewing temperature data for correct readings, writing step by step procedures for the analysis of each parameter in your NPDES discharge permit

Accuracy - how close the measured result is to the true value for a specific test;

Bias - results that are consistently greater than or less than the true value due to systematic errors in the procedure;

> Example: The barometer used to calibrate the dissolved oxygen probe reads higher than the true barometric pressure. This results in consistently high dissolved oxygen readings during the BOD_5 analysis

Precision - a measure of the agreement of results between two or more measurements of the same sample collected during the same sampling event;

Data Quality Objectives (DQOs) - the decision made as to how accurate analytical results must be for regulatory and/or process control purposes;

> Example: A laboratory is required to analyze for Total Suspended Solids on the

Influent sample as part of their NPDES discharge permit. The plant superintendent requests Volatile Suspended Solids analysis on the Influent for process control purposes. This lab might set a DQO of 10% RPD for Total Suspended Solid replicates for reporting purposes and a DQO of 20% RPD for Volatile Suspended Solid replicates for control purposes

Random Error - Error that results from inconsistent sampling and/or analytical methods;

Examples: (1) Sampler collects a sample and allows it to settle while filling out the sample collection record. The sampler then pours a portion of the sample into a composite sample bottle without mixing it, thus the sample added is not representative of the sample collected or, (2) while analyzing for Total Suspended Solids, a small twig is in the sample being filtered. By failing to remove this twig, the analyst is introducing random error into the analysis

Systematic Error - Error that results from improper calibration and/or the consistent incorrect use of equipment or procedures;

Examples: (1) In preparing calibration standards for ammonia analysis, laboratory personnel routinely use a graduated cylinder instead of a Class A volumetric flask to adjust the final volume to 100 mL. By doing this, systematic error may be introduced into the analysis or, (2) an uncertified thermometer was used in a drying oven used for Total Suspended Solids analysis and even though the thermometer read 104 °C, the actual oven temperature was 112 °C.

Representativeness - measures how well the results actually reflect the sample site you are trying to monitor. Representativeness is achieved by making sure proper sampling techniques are used, using the correct analytical procedures, meeting sample holding times, and the analysis of sample duplicates;

Matrix - what the sample consists of (drinking water, wastewater, sludge, soil, etc.);

Reagent Blank - a measured volume of laboratory water that is treated the same as the samples to check for water quality, reagent purity, glassware cleanliness, or other possible sources of contamination;

Reagent Water – may be deionized, distilled, reverse-osmosis, etc., as long as the water quality meets the criteria defined under Reagent Water Quality on page 8.

Laboratory Control Sample - a measured volume of laboratory water to which a known amount of the analyte being tested for is added followed by the same treatment given to the samples **(This may also be referred to as a Laboratory Fortified Blank)**;

Calibration Check Standard - a standard used to check the calibration of an instrument between complete calibration curve determinations;

Example: The laboratory analyst calibrates the ammonia apparatus using 0.1 mg/L, 1.0 mg/L and 10. mg/L standards and obtains an acceptable slope of 97%. Halfway through the analysis of 14 samples, the analyst analyzes a 3.0 mg/L Calibration Check Standard to make sure the ammonia apparatus is still in calibration. The 3.0 mg/L standard gives a result of 3.08 mg/L that is within 5% of the true value, thus the analyst continues analysis with the knowledge the ammonia apparatus is still in calibration. NOTE: Although these may not be the exact standards used in your laboratory, any standards used should be in factors of ten and bracket the expected sample concentration. See the individual procedures for specific details.

Duplicate - in this document, a duplicate is the smallest number of replicates (two) analyzed to check for precision. Another definition commonly used is: two samples collected at the same place at the same time. The analysis of this type of duplicate checks for representativeness;

Replicate(s) - two or more analyses for the same parameter taken from a single sample;

Laboratory/Quality Control Standard - a purchased standard certified to have a known concentration of analyte in it. Analysis of this type of standard is used to check for accuracy and bias;

Matrix Spike - a measured volume of sample to which a known amount of the analyte of interest is added followed by the same treatment given to all other samples in the analytical run;

Standard Operating Procedure (SOP) - a written document for each analytical test performed in the laboratory documenting in step-wise detail the entire procedure followed for the analysis of samples in the laboratory

II. Quality Control Measures

Laboratory Cleanliness

The laboratory is to be kept clean and organized at all times. The room temperature should be kept as constant as possible. Appropriate actions are taken to maintain air quality.

Personnel Training

Training for all personnel involved with laboratory analysis must be documented showing that they are capable of meeting the Data Quality Objectives (DQOs). Initial performance can be demonstrated through the use of Quality Control Standards and internal or external split samples. Continuing training on a regular basis should be provided to help maintain competence of analytical skills.

Equipment Maintenance

Files are maintained for each piece of laboratory equipment. These files contain the

operating manual, a preventative maintenance schedule, and a record of any maintenance and repairs performed. The maintenance and repair record is to contain the following information: nature of problem, date of repair, maintenance/repair performed, person performing maintenance/repair, and cost, if any. These records will help the laboratory personnel determine if results were affected by an instrument malfunction. As part of the routine equipment maintenance, analytical balances are serviced annually and dissolved oxygen and ammonia probe membranes may need to be replaced every two to four weeks unless readings become unstable, then these membranes are replaced immediately.

Analytical Reagents

All chemicals and reagents used in the laboratory are analytical grade or better (such as ACS). Upon receipt in the laboratory, each chemical is marked in permanent ink with date received on the label and when opened for the first time. Any reagents or solutions prepared in the laboratory must have a label with the date prepared and by whom. Because labels are replaced, this information is also recorded in a permanent record as part of the three-year record keeping requirement. Chemicals and reagents are stored away from direct sunlight and, if necessary, refrigerated to prevent deterioration. If refridgerated, chemicals should be brought to room temperature before aliquots are measured. Stock chemicals and reagents are transferred to a clean container prior to weighing, pipetting, etc., to prevent contamination. All solutions or reagents are discarded and ordered or prepared fresh after being opened one year unless the analytical method specifies a shorter time period for replacement. All chemicals are stored in a safe manner and segregated by hazard type. Those hazard types commonly found in a wastewater laboratory are:

Health - identified with Blue on the label
Reactive - identified with Yellow on the label
Corrosive - identified with White on the label
Flammable - identified with Red on the label
General - identified with Green or Orange on the label.

All flammable chemicals are to be stored in a fireproof cabinet and strong acids and bases are stored separately from each other. In the event, a chemical has stripe marks on the colored hazard code label, this is a warning not to store it with chemicals of the same colored hazard code. An example being a chemical with a striped yellow hazard code label is to be stored separately from chemicals with a yellow hazard code.

A Material Safety Data Sheet (MSDS) is to be on file and accessible for all purchased chemicals and reagents used by the laboratory personnel.

Reagent Water Quality

Reagent grade water used for chemical analysis is produced in the laboratory using distillation, reverse osmosis or ion exchange and meets the following specifications:

Resistivity, megohm-cm at 25 degrees C = >1
Conductivity, Fmho/cm at 25 degrees C = <1

SiO_2, mg/L	= < 0.1

In addition, if the reagent grade water is used for bacteriological analysis, it must meet the following additional specifications:

pH	= 5.5 - 7.5 S.U.
Total organic carbon	= < 1.0 mg/L
Heavy metals, single (Cd, Cr, Cu, Ni, Pb, and Zn)	= < 0.05 mg/L
Heavy metals, total	= < 0.10 mg
Ammonia/organic nitrogen	= < 0.10 mg/L
Total chlorine residual	= < 0.01 mg/L

Only freshly prepared reagent water is used for ammonia analysis to prevent contamination from ammonia in the air. Dilution water for BOD_5 analysis is stored in light shielded containers, sealed in such a manner to allow a free exchange of air without contamination. (Examples: cotton plugs, loose fitting container lids) Reagent water for other laboratory tests is stored in tightly stoppered glass containers or withdrawn fresh from the water purification unit.

Labware Cleaning

After each use, glassware and plastic ware is washed with detergent, rinsed with tap water followed by a thorough rinse with reagent water. After drying, it is stored in a cabinet. There are appropriate glassware cleaning procedures for specific tests, for example, glassware used for total phosphorus analysis is washed in non-phosphate detergent, acid-washed after each use and kept separate from other laboratory glassware. (The best choice would be using a detergent that is both ammonia- and phosphate-free, thus eliminating having different detergents for different procedures.) Care must be taken to store BOD_5 bottles dry to prevent the growth of bacteria or algae in the bottle. The BOD_5 siphon tubing should be cleaned monthly with dilute bleach solution (25 mL bleach / Liter of reagent water) inspected daily for growth and replaced or cleaned more often if any growth is observed. All containers used to store reagent water are inspected for growth or other signs of contamination prior to withdrawing water from them. If growth or signs of contamination are observed, the reagent water is discarded and the container cleaned with dilute hydrochloric acid followed by rinsing with tap water and a thorough rinsing with reagent water.

Instrument Calibration

The pH meter, dissolved oxygen meter, and ammonia ion selective electrode are calibrated each day they are used. If they are used throughout the day, a calibration check is repeated if more than two hours has passed following the initial calibration. Temperature of incubators and refrigerators are measured on thermometers with their bulbs immersed in liquid, **see note below**. Each day the temperature of the incubators and refrigerators are recorded on a log sheet taped to the equipment or in a logbook. The temperatures of the drying oven(s), muffle furnace and bacteria incubation baths are recorded on the bench sheet or in a logbook when these pieces of laboratory equipment are used for analysis. The temperature of the autoclave is read using a maximum reading thermometer and this reading recorded on the bench sheet or in a logbook. When equipment temperatures read outside the required range, the equipment thermostats are

adjusted and this adjustment is recorded on the log sheets, bench sheets, or in the logbook.

Thermometers used in the laboratory are calibrated annually against a thermometer traceable to an NIST (National Institute of Standards and Technology) certified thermometer. The NIST thermometer must have been certified within the past five years. Any correction factors associated with the NIST certified thermometer are recorded on the thermometer calibration log sheet and any correction factors associated with the laboratory thermometer(s) are noted on a tag attached to the thermometer. If the liquid column in the thermometer becomes separated, the thermometer in no longer accurate and must be replaced. An alternative to calibrating laboratory thermometers is to purchase factory certified thermometers traceable to NIST thermometers. These thermometers are sealed in a clear container with the bulb in a medium appropriate to the equipment temperature being measured. Each thermometer comes with a unique serial number, a certificate of NIST traceability, and the required re-certification and/or expiration date.

Note: Thermometers are calibrated for total immersion or partial immersion. Those calibrated for partial immersion must be immersed only to the depth of the etched circle around the stem of the thermometer just below the thermometer scale readings. Those calibrated for total immersion must be completely immersed in the matrix being measured.

Analytical balances are zeroed daily and the calibration checked at least monthly. One of these weights is in the milligram and one in the gram range. Documentation of the daily zeroing and monthly weight checks are recorded in a logbook. If the balance readings are not within 0.5 mg of the certified weight, a certified technician must service the balance.

All records relating to instrument calibrations, thermometer calibrations, and temperature logs must be retained a minimum of three years.

Quality Control Analysis

Analysis of blanks, replicates, standards and matrix spikes are performed on a routine basis and at the frequency listed in **Table 1**. Blanks are processed through the entire analytical procedure including the addition of any preservatives the associated samples have in them. The results from the reagent blanks are treated as specified in each analytical method. Results from reagent blanks, replicates, standards, and matrix spike analyses are recorded on the daily bench sheets and on their respective control charts.

Table 1
Quality Control Sample Frequency

Test	Calibration	Known Standards	Blanks	Replicates	Matrix Spikes
BOD_5	Calibrate DO meter on each analysis day	1 GGA Standard with each new batch of dilution water	*Daily	*Daily	NA
TSS	Daily - Zero Balance Monthly - Checked with (2) certified weights Annually - Serviced by certified technician	NA	*Daily	*Daily	NA
Ammonia-Nitrogen	*Daily	*Daily	*Daily	*Daily	*Daily
Total Phosphorus	*Daily Alternatively, establish a calibration curve and verify *Daily with a known standard	*Daily	*Daily	*Daily	*Daily
pH	*Daily	NA	NA	*Daily	NA
Residual Chlorine	Initially, and with each reagent change or if Known Standard exceeds acceptance range	Weekly	*Daily	Weekly	NA
Bacteriological	NA	NA	*Daily	Weekly	NA

GGA = Glucose-Glutamic Acid *Daily = Each time analysis is performed

Blind Standard

A blind standard is a sample obtained from an outside source whose concentration is validated and known by the supervisor but unknown to the laboratory personnel. This sample is analyzed in the laboratory and the result submitted to a supervisor. If the laboratory result is within acceptable levels, no further action is necessary. If the laboratory result is outside the acceptable levels, appropriate measures are taken to trouble shoot the problem to prevent continuing problems within the laboratory. Blind samples are analyzed every six months as part of the quality assurance/quality control program.

Calibration Check Standard

A standard prepared or purchased by the laboratory with a known/validated

concentration. This type of standard is used to verify a previously established calibration curve and/or the accuracy of the analytical system. This standard should not be prepared from the same source as the calibration standards. This standard is also referred to as a "second source" standard, meaning purchased or prepared from a different lot or manufacturer.

Reference Samples
A sample obtained from an approved external source whose concentration is unknown to the laboratory. The Indiana Department of Environmental Management (IDEM) sends these to many Indiana wastewater laboratories as part of the Mayflower Program. Results from reference samples are submitted to the provider to determine whether the results produced by the laboratory are within the acceptable range. If the submitted results are outside the acceptable range, the laboratory is contacted as to what corrective action is required.

III. Calibration

Much emphasis is placed on the analysis of known standards, reference samples, blanks, replicates, and blind standard to document accuracy, bias, and precision, but the importance of the calibration curve may be overlooked during the analytical procedure. Some instruments come with pre-programmed calibration curves, some analysts hand-draw their calibration curves or use scientific calculators to construct the curve, and the use of computer programs is increasingly being used.

Simply put, the calibration curve shows the relationship between the instrument and/or probe response to different concentrations of analyte. The following guidelines are applied to this relationship to ensure the reporting of accurate results.

Appropriate number of standards
Calibration curves must be established using a least three standards and a blank - it is not usual practice to have a blank as part of the ammonia probe calibration. After ammonia probe calibration is established using a minimum of three standards, a blank is analyzed following the same procedure used to analyze samples. The result from the blank analysis is used to determine where the calibration curve intersects the x or y-axis in addition to checking for possible contamination problems.

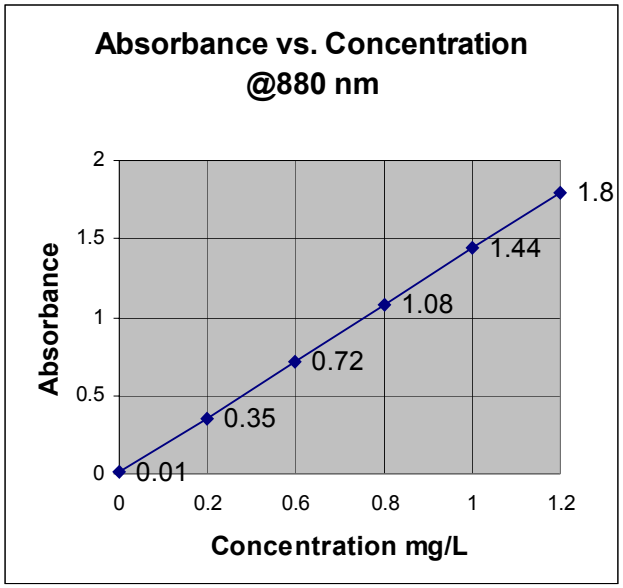
Keeping it simple - linear is the key
Most of the instruments found in a wastewater laboratory respond to analyte concentrations in a linear manner. When the instrument does not follow the linear response, it is usually at the low and high extremes of the analytical curve. This usually results from overloading the instrument's detector at the high end and instrument noise and lack of sensitivity at the low end of the analytical curve. The simplest method to generate a calibration curve is through the use of a linear regression formula and the most defensible way to do this is to plot analyte concentration on the x-axis (horizontal) and the instrument response on the y-axis (vertical).

How to handle the calibration blank

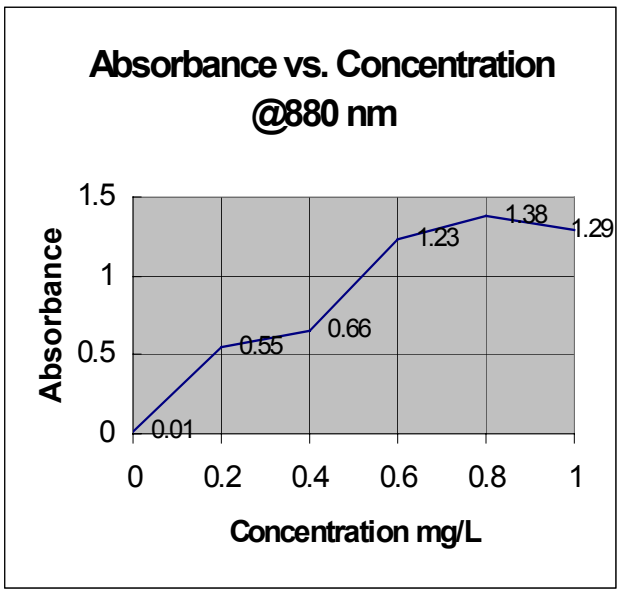
Unless the calibration algorithm (mathematical formula) you are using has a perfect fit for the calibration curve, i.e., a correlation coefficient of 1.000..., you must decide what to do about the calibration blank result. A good rule to follow is: if you can adjust your instrument/probe to read zero in the presence of the blank, then the zero point should be included in the calibration curve. Including the calibration blank (zero standard) is generally acceptable for colorimetric procedures that use a spectrophotometer to measure the response to the standards and samples.

Forcing curves through zero

The practice of manipulating the data to obtain a y-intercept equal to zero usually results in biasing data that falls close to the lower end of the calibration curve. This "artificial" manipulation may result in losing important information relative to samples with low analytical signals as well as losing information relative to the limit of detection for that day's calibration. Even when the analytical method specifies using a blank as part of the calibration curve, forcing the intercept to read zero is not specified. Having said this, it is noted that the Standard Methods procedure for total phosphorus makes the following statement, "Plot absorbance vs. phosphate concentration to give a straight line passing through the origin". As a result, while this practice is discouraged in the laboratory, it is allowable to do this for total phosphorus calibrations.



Calibration 1



Calibration 2

Checking the calibration curve for accuracy

In the calibration graphs above, most laboratory analysts would agree that Calibration 1 is much better than Calibration 2. Methods usually read "plot concentration vs. response", so without evaluating the calibration curves, the quality of data may suffer. One means of determining the validity of a calibration is the correlation coefficient. When the correlation coefficient is at least 0.995, the calibration curve is usually considered acceptable. When the correlation coefficient is less than 0.995, the reason should be investigated, corrective action taken, and a new calibration curve established. Any scientific calculator capable of performing linear regression should display the correlation coefficient when the proper keypad entry sequence is followed. In the graphs above, Calibration 1 has an acceptable correlation coefficient of 0.9996 while Calibration 2 has an unacceptable correlation coefficient of 0.93881.

An additional means of checking the calibration curve is to use the calibration equation (e.g., slope and intercept) to convert the instrument response for the calibration standards into their respective concentrations based on the established calibration curve. There should be "reasonable" agreement between the "true" concentrations (e.g., the concentrations prepared by the analyst) and the concentrations calculated using the calibration curve. Depending on the individual laboratory's DQOs, the calculated concentrations should be within 5 - 10% of the "true" concentrations.

Using the slope and intercept to evaluate the data used to establish Calibration 1 and 2 above, **Table 2** reflects why Calibration 1 is acceptable and Calibration 2 is unacceptable.

Table 2
Calibration 1 vs Calibration 2 Calculated Concentrations

"True Value	0.00	0.20	0.40	0.60	0.80	1.0
Calibration 1 Calculated	0.0048	0.19	0.40	0.60	0.80	1.0
Calibration 2 Calculated	-0.12	0.28	0.36	0.78	0.89	0.82

Establishing the calibration range correctly
Just as we do not use a sledgehammer when installing trim around a doorway or a claw hammer to bust up large areas of concrete, we should establish a calibration curve range appropriate for the samples being analyzed. For example, when analyzing for ammonia-nitrogen, if historical data shows that sample concentrations are between 0.05 and 14.0 mg/L, we don't calibrate from 1.0 to 500. mg/L. The most accurate results are obtained when the results from the samples with unknown concentration are close to the known standards used to construct the calibration curve.

A well constructed calibration curve should contain evenly spaced standards (at least three) with the realization that the more standards used, the better the quality of data generated. **When quantifying samples, a full calibration curve is always used**. The use of a calibration check standard or quality control standard should only be used to verify the calibration curve, but by itself, never used to for quantifying samples.

Instruments with Pre-programmed Calibrations
Some of the commercially available spectrophotometers are sold with "pre-programmed" calibration curves for common wastewater tests including chlorine residual and total phosphorus. Single parameter spectrophotometers are generally referred to as colorimeters. If reference samples produce unacceptable results, recalibrating the instrument should be considered as a corrective action. Consult the manufacturer's instructions for a user-entered calibration.

Calibration Curves Hand-drawn by the Analyst
The practice of the laboratory analyst hand-drawing what they believe to be the "best-fit" for the calibration data is strongly discouraged. The use of self-initiated scaling techniques for the calibration graph in addition to the how one determines the "best-fit" for the curve makes the end results subjective in nature. The line you draw as "best-fit" may not be the line that best fits the data.

Using Scientific Calculators or Computer Spreadsheets to Define the Calibration Curve
An acceptable standard practice to generate the calibration curve is to use the linear regression function of a scientific calculator or a computer spreadsheet. The use of this standardized statistical procedure will produce consistent equations for the "best-fit" line, thus eliminating the guesswork or bias with the hand-drawn line.

IV. Level of Detection (LOD)

One of the key questions a laboratory analyst should ask is "at what point can I distinguish a sample result from a blank result for samples with low concentrations of analyte?" This concentration has historically been designated the Method Detection Limit (MDL) but in this document will be designated the Level of Detection (LOD). When your results approach the LOD, precision is usually reduced due to instrument noise and other variables. Because of this, it is important for the analyst to understand whether there is analyte present or not.

The determination of the LOD is required for each parameter reportable to IDEM, exceptions being LODs for Biochemical Oxygen Demand (BOD_5), Total Suspended Solids (TSS), pH, and parameters associated with solids (sludge). BOD_5 and TSS LODs are discussed later in this section. Laboratories usually determine LODs by spiking samples prepared with laboratory reagent water. However, the method of using laboratory reagent water does not take into effect how the wastewater matrix could influence the LOD results. It is recommended that each LOD for your facility be determined by adding the appropriate amount of spike to a wastewater effluent sample. As each wastewater treatment plant effluent contains different concentrations of ammonia-nitrogen and/or total phosphorus, the examples given below illustrate the procedure to follow when the laboratory reagent water matrix is used for the LOD determinations. The simplest procedure for determining the LOD is as follow:

1. Determine the spike concentration that approximates the LOD

2. Prepare seven or eight replicates of laboratory reagent water or effluent sample spiked at the appropriate level

3. Analyze the replicates

4. Calculate the LOD

5. Perform the "4-point" check of the LOD (found on page 18)

6. Repeat the LOD determination as needed

How to Choose the Appropriate Spike Level

The LOD is an estimate for the lower level of the calibration curve, thus the best spiking level is 1 - 5 times the estimated detection level. Standard Methods and the EPA procedure found in 40 CFR, Part 136 explain this procedure in detail.

For ammonia-nitrogen, a spike concentration between 0.05 and 0.25 mg/L is usually appropriate for the LOD determination.

The spike concentration is equal to the ammonia-nitrogen concentration in the samples you are analyzing when determining the LOD. To prepare seven or eight

replicates at the 0.05 mg/L concentration level, you would add 5.0 mL of a 10. mg/L ammonia-nitrogen standard to a 1 liter volumetric flask using ammonia free reagent water to bring the volume in the 1 liter flask to the 1 liter mark. After thoroughly mixing the contents of the 1 liter flask, the seven or eight replicate samples to be used for the determination of the LOD are measured out.

If determining the LOD using seven or eight replicates at a concentration of 0.25 mg/L, you would add 25.0 mL of a 10. mg/L ammonia-nitrogen standard to a 1 liter flask and add ammonia free reagent water to the 1 liter mark. Mix completely and measure out the seven or eight replicates to be analyzed.

For total phosphorus, a spike concentration between 0.01 and 0.05 should result in a valid LOD determination.

The spike concentration is equal to the total phosphorus concentration in the samples you are analyzing when determining the LOD. To prepare seven or eight replicates at the 0.01 mg/L concentration level, you would add 10.0 mL of a 1.0 mg/L total phosphorus standard to a 1 liter volumetric flask and use laboratory reagent water to bring the volume in the 1 liter flask to the liter mark. After thoroughly mixing the contents of the 1 liter flask, the seven or eight replicate samples used for the determination of the LOD are measured out.

If determining the LOD using seven or eight replicates at a concentration of 0.05 mg/L concentration, you would add 5.0 mL of a 10. mg/L total phosphorus standard to a 1 liter flask and add laboratory reagent water to the 1 liter mark. Mix completely and measure out the seven or eight replicates to be analyzed.

Calculation the Level of Detection (LOD)

LOD = Standard Deviation x Student's t-value

You must use the following information when calculating the LOD:

1. The standard deviation for the seven or eight replicates analyzed;

2. The correct Student's t-value; and

3. All significant figures.

Standard Deviation - most scientific calculators will calculate the standard deviation for the data set. The **key** item to remember is, after entering the data for the seven or eight replicates following the entry sequence specified by the manufacturer of your scientific calculator, you must calculate the **sample** standard deviation (usually designated "s_{n-1}" or "Fn-1" on the calculator) **not the population** standard deviation (usually designated as "s" or "Fn" on the calculator).

Student's t-value - below is a portion of the **Student's t-value Table** for use when calculating LODs. A more complete version of the table can be found in 40 CFR 136, Appendix B.

# of replicates	Student's t-value
6	3.365
7	3.143
8	2.998
9	2.896

The Student's t-value for eight and nine replicates is given in the event you use more than seven replicates in the LOD determination. The Student's t-value for six replicates is included in the event one of the replicates does not "fit" with the other replicates or one of the replicate samples is lost due to laboratory error or accident.

Significant figures - these must be carried throughout the entire LOD calculation procedure followed by rounding to the number of significant figures used for reporting results. It is permissible to round the calculated LOD value up to the nearest decimal place, i.e., if the LOD calculates to 0.15 mg/L, it is acceptable to round this value to 0.2 mg/L when results are only reported to one significant figure to the right of the decimal point.

How Often to Determine the LOD

The frequency for determining the LOD should be specified in the Standard Operating Procedure (SOP) for each of the NPDES required analytical tests performed in the wastewater laboratory. Often this is annually, but other circumstances may require more frequent LOD determinations. These may include whenever a new analyst begins producing data, a change in the analytical system (replacement light bulb in spectrophotometer or the replacement of the ammonia or dissolved oxygen probe), or a new procedure being used in the laboratory, to name a few. A laboratory may choose to incorporate the LOD determinations on an annual schedule to make sure their results are up to date and usable.

Checking Your LOD Determinations ("4-Point" Check)

The LOD calculated in the laboratory can be checked using the following criteria:

1. Was the concentration of the replicate samples analyzed to determine the LOD greater than 10 times the calculated LOD? Is yes, the replicate sample concentrations were too high and a lower sample concentration needs to be prepared and the LOD determined again;

2. Is the calculated LOD higher than the concentration of the replicate samples

used in the determination of the LOD? If yes, the replicate sample concentrations were too low and a higher sample concentration needs to be prepared and the LOD determined again;

3. Is the calculated LOD greater than the discharge permit limits specified in your wastewater permit? Even the lowest discharge permit limits should be significantly greater than the LODs calculated in the wastewater laboratory. If the LOD's calculated in your laboratory are higher than your discharge permit limits, you need to evaluate the entire analytical procedure and correct any problems causing high LOD values;

4. Are the results obtained from the analysis of the replicate samples analyzed to determine the LOD reasonable when compared to the actual concentration of the replicates analyzed? For example, if the concentration of seven replicates used to determine the LOD for ammonia-nitrogen was 0.15 mg/L and the calculated mean (average) of the seven replicates was 0.63 mg/L, the results are questionable. A good rule of the thumb to follow is that the mean result of the replicates used for the LOD determination should be within 20 percent of the prepared/known concentration. Since the concentration of the replicates used for LOD determinations is at the lower end of the useable concentration range in the wastewater laboratory, any reference made in the Standard Operating Procedure as to the treatment of reagent blanks during calculations should be followed.

The following equations are useful when evaluating the calculated LOD:

Calculated LOD < **Spike Level** < 10 times the calculated LOD

Mean result (mg/L) of the 7 replicates = Prepared concentration (mg/L) ± 20%

BOD_5 Level of Detection

It is not required to perform a LOD for BOD_5 analysis due to the requirement that BOD_5 samples must have a 2.0 mg/L oxygen depletion to be considered reportable. Because of this and that BOD_5 analysis is usually performed in a 300 mL sample bottle, the LOD for a 300 mL sample is by definition 2.0 mg/L. (2.0 mg/L required oxygen depletion)(300 mL sample/300 mL bottle) = 2.0 mg/L

Table 3 demonstrates how using different sample volumes affect the LOD:

Table 3

Highest Sample Volume Used	BOD_5 LOD
300 mL	2.0 mg/L
200 mL	3.0 mg/L
100 mL	6.0 mg/L
75 mL	8.0 mg/L
50 mL	12. mg/L
10 mL	60. mg/L

Example: Three dilutions were analyzed on the plant effluent with the following results:

Dilution 1	volume used is 150 mL	BOD_5 = 20 mg/L
Dilution 2	volume used is 100 mL	BOD_5 = 22 mg/L
Dilution 3	volume used is 75 mL	BOD_5 = 15 mg/L

Assuming all dilutions had an oxygen depletion of 2.0 mg/L and a final DO of greater than 1.0 mg/L, the reportable BOD_5 is 19 mg/L.
(20 + 22 + 15) = 57 ÷ 3 = 19 with a LOD of 4.0 mg/L based on the highest sample volume being 150 mL.

If the dilutions listed in the example above were used on a separate sample and all three samples failed to have the required oxygen depletion of 2.0 mg/L, then the reported result would be #4.0 mg/L BOD_5.

In the event all of the dilutions analyzed for BOD_5 have too much oxygen depletion, e.g., there is less than (<) 1.0 mg/L oxygen remaining after the five day incubation period, the analyst is to use the lowest sample dilution used to calculate the result and report the result as greater than or equal to ($) the calculated value and decrease the sample volumes used for BOD_5 analysis on this sample type until the data indicates higher sample volumes can be analyzed. Using the sample volumes in the example above and given the following information, the BOD_5 calculation gives a result of $ 31.

Initial dissolved oxygen reading for 75 mL sample volume = 8.4 mg/L
Final dissolved oxygen reading after 5 day incubation at 20^o C = 0.7 mg/L
Note that reading is less than (<) 1.0 mg/L dissolved oxygen
Dissolved oxygen depletion = 7.7 mg/L

$$BOD_5 = \frac{\text{(300 mL } BOD_5 \text{ bottle sample volume)(7.7 mg/L oxygen depletion)}}{\text{75 mL sample volume used}} = \$31 \text{ mg/L}$$

Total Suspended Solids (TSS) Level of Detection

Similar to the BOD_5 test, the LOD for TSS is based on a minimum retention requirement of 1 mg of residue on the filter used for the TSS determination. Thus, if you filtered a 1 liter sample, you can "detect" 1 mg/L. Consequently, the LOD is dependent on the sample volume filtered - see **Table 4**

Table 4

Sample volume filtered	TSS LOD
500 mL	2.0 mg/L
250 mL	4.0 mg/L
100 mL	10 mg/L
50 mL	20 mg/L
25 mL	40 mg/L

A Sample LOD calculation for Ammonia-nitrogen

When reading the manufacturer's instruction manual for the ammonia-nitrogen probe, the lab analyst read the statement that the probe could detect 0.05 mg/L ammonia-nitrogen. The analyst thus prepared one liter of 0.1 mg/L ammonia-nitrogen solution as this met the criteria of preparing a sample in the range of 1 - 5 times the estimated Level of Detection. Analysis of seven 100 mL replicates measured from the one liter of 0.1 mg/L ammonia-nitrogen solution gave the following results;

Replicate 1	0.104
Replicate 2	0.093
Replicate 3	0.096
Replicate 4	0.1
Replicate 5	0.112
Replicate 6	0.113
Replicate 7	0.088
Mean	**0.1008571**
Std. Dev.	**0.0094239**
Student's t-value	**3.143** (from chart on page 18)
LOD	**0.0296193** (Standard Deviation x Student's t-value)

The laboratory's ammonia-nitrogen method specifies reporting ammonia-nitrogen results to one significant figure to the right of the decimal point, thus the LOD is rounded to 0.03 mg/L

The analyst then checks to make sure the LOD meets the established criteria:

1. Is the concentration of the replicate samples analyzed greater than 10 times the calculated LOD?
   No (0.1 mg/L ÷ 0.03 mg/L) = 3.33 **This criteria is met**

2. Is the LOD higher than the concentration of the replicate samples analyzed?
   No (0.03 mg/L < 0.1 mg/L) **This criteria is met**

3. Is the LOD higher than the discharge permit level for ammonia-nitrogen? The NPDES permit for this facility specifies an ammonia-nitrogen discharge limit of 2.2 mg/L.
   No (0.03 mg/L < 2.2 mg/L) **This criteria is met**

4. Is the mean concentration resulting from the analysis of the seven replicates within 20% of the prepared concentration?
   Prepared concentration = 0.1 mg/L
   Mean concentration from analysis = 0.1008571
   (Mean concentration ÷ Prepared concentration) x 100 = 100.8571%
   Yes - Mean concentration is within 0.8571% of the prepared concentration
   This criteria is met

It is important to note that many digits were carried through the LOD calculation but the final LOD was rounded to the number of significant figures called for in the laboratory's method for ammonia-nitrogen.

Although the example above will not reflect the results obtained in your wastewater laboratory, they are included as an aid in your calculation of LODs. By following the manufacturers's instructions for calculating the mean and standard deviation on your scientific calculator, you should obtain the mean and standard deviation results above. If this is not the case, you need to perform the calculations again.

V. Evaluating Precision

There are numerous methods by which laboratory precision can be measured. The method outlined below is suggested so that your laboratory can evaluate precision the first time replicates are analyzed.

Replicate analyses are performed at the frequency set forth in **Table I**. The difference between the results of replicate analyses and the mean (average) of the replicates is used to calculate the precision (reproducibility) of the analytical method.

Step 1 - Calculate the range (R) for each set of replicate samples

Range (R) = *(Sample Result) - (Replicate Result)*
Where:*math calculation* = absolute value of the "math calculation"

The absolute value simply means that negative values are expressed as positive

values, thus *14 - 18* = 4 rather than -4. One can think of Range as the difference between two replicate sample results always resulting in a positive value.

Step 2 - Calculate the Average "mean" (0) for each set of replicate samples

$$\text{Mean } (0) = \frac{\text{(Sample Result) + (Replicate Result)}}{2}$$

Step 3 - Calculate the Relative Percent Difference (RPD)

$$\text{RPD} = \frac{\text{Range (R)}}{\text{Mean } (0)} \times 100$$

When using Relative Percent Difference (RPD), the following Control Limits and Warning Limits are used to evaluate precision in the wastewater laboratory:

1. If the mean (0) of the replicates is less than or equal to (#) five times the LOD, the LOD is the control limit and two-thirds (or 0.67 times) the LOD is the warning limit

2. If the mean (0) of the replicates is greater than (>) five times and less than (<) 20 times the LOD, the control limit is 25% RPD and the warning limit is 16.7% RPD

3. If the mean (0) of the replicates is greater than or equal to ($) 20 times the LOD, the control limit is10% RPD and the warning limit is 6.7% RPD.

For many analyses, after establishing the LOD for a given parameter, the three ranges listed above for evaluating precision in the laboratory, e.g., #five times the LOD, > five to < 20 times the LOD and $20 times the LOD do not change until a new LOD is calculated. For BOD_5 and TSS analyses, that have a pre-defined LOD, the analyst needs to keep in mind the sample volumes used during analysis to accurately evaluate whether the precision between the sample and the replicate sample are acceptable. **Table 5** illustrates how sample volume used for BOD_5 replicate analysis affects the three ranges for calculating precision within the laboratory and **Table 6** displays the corresponding information for Total Suspended Solids (TSS) replicate analysis. It is noted that should the mean result for the sample and sample replicate give a result less than the LOD, calculation of precision is not necessary. The reason for this being that at very low concentrations, precision or reproducibility can be greatly influenced by instrumental background noise. Thus any precision number calculated may reflect this instrument noise rather than the capabilities of the analyst or the analytical procedure.

For BOD_5 analysis, precision and bias is also evaluated through the analysis of the glucose-glutamic acid (GGA) check sample. Standard Methods states the recovery of this primary mixed standard should be 198 ± 30.5 mg/L. Analytical results consistently in this acceptable range demonstrate good dilution water quality, good seed effectiveness, and good technique by the analyst. Results consistently above or below the 198 mg/L value indicate bias. When this occurs, the laboratory should investigate the cause and take corrective action to eliminate the bias.

Table 5
The Effects of Sample Volume Used on Precision Ranges for BOD_5 Analysis

Greatest Sample Volume (mL) Used for Sample Replicates	LOD mg/L	Precision Range 1 LOD to #5x LOD		Precision Range 2 > 5x LOD to < 20x LOD		Precision Range 3 $20x LOD	
		Mean of Replicates mg/L	CL = mg/L WL = mg/L	Mean of Replicates mg/L	CL = RPD WL = RPD	Mean of Replicates mg/L	CL = RPD WL = RPD
300	2.0	2.0 to #10.	2.0 1.3	>10. to <40.	25% 16.7%	$40.	10% 6.7%
200	3.0	3.0 to #15	3.0 2.0	>15. to <60.	do	$60.	do
150	4.0	4.0 to #20.	4.0 2.7	>20. to <80.	do	$80.	do
100	6.0	6.0 to #30.	6.0 4.0	>30. to < 120	do	$120	do
50	12.	12. to #60.	12 8.0	>60. to <240	do	$240	do
25	24.	24. to #120	24. 16.	>120 to <480	do	$480	do
10	60.	60. to #300	60. 40.	>300 to <1200	do	$1200	do

CL = Control Limit WL = Warning do = same as above

Table 6
The Effects of Sample Volume Used on Precision Ranges for TSS Analysis

Greatest Sample Volume (mL) Used for Sample Replicates	LOD mg/L	Precision Range 1 LOD to #5x LOD		Precision Range 2 > 5x LOD to < 20x LOD		Precision Range 3 $20x LOD	
		Mean of Replicates mg/L	CL = mg/L WL = mg/L	Mean of Replicates mg/L	CL = RPD WL = RPD	Mean of Replicates mg/L	CL = RPD WL = RPD
1000	1.0	1.0 to #5.0	1.0 0.7	>5.0 to <20.	25% 16.7%	$20.	10% 6.7%
500	2.0	2.0 to #10.	2.0 1.3	>10. to <40.	do	$40.	do
250	4.0	4.0 to #20.	4.0 2.7	>20. to <80.	do	$80.	do
100	10.	10. to #50.	10. 6.7	>50. to < 200	do	$200	do
50	20.	20. to #100	20 13.	>100 to <400	do	$400	do
25	40.	40. to #200	40. 27.	>200 to <800	do	$800	do
10	100	100 to #500	100 67.	>500 to <2000	do	$2000	do

CL = Control Limit WL = Warning do = same as above

VI. Evaluating Accuracy

As defined in the Introduction of this document, accuracy measures how close the laboratory result is to the "true" value. Not only is it important for the laboratory to report accurate and legally defensible results to IDEM to help that agency control any adverse effects the treated wastewater effluents may have on the water quality and biological community of the receiving waters, but accurate test results are also needed by the wastewater treatment plant personnel for proper process control.

The two samples most commonly used in the wastewater laboratory to determine accuracy are the Laboratory and/or Quality Control Standard and the Matrix Spike.

Laboratory/Quality Control Standard
As defined in the introduction, this is a purchased standard that comes with a certificate of analysis listing the "true" value. In addition to the "true" value, the certificate usually indicates how close the laboratory result must be to the "true" value to be acceptable. An example follows:

Standard	Units	Certified Value	Acceptance Limits
Ammonia-Nitrogen	mg/L	8.36±0.13	6.59 - 10.4
Total Phosphorus	mg/L	8.60±0.091	6.35 - 9.75

Thus, if the measured laboratory result for the Ammonia-Nitrogen = 7.89 mg/L, the result is acceptable with an accuracy of 94.4%
(Measured result ÷ "True" Value)(100) = Accuracy/Percent Recovery

The Laboratory/Quality Control Standard is a useful means of checking the accuracy of the calibration standards used to prepare the calibration curve in addition to checking for bias (systematic error) in the analytical procedure.

Matrix Spike Sample
Percent recovery data calculated from the analysis of spiked samples is used to determine the accuracy, or bias, of the analysis. Spiking a sample is the process of adding a measured amount of a known concentration of the targeted analyte to a measured amount of the sample. Samples are spiked prior to being processed through any preliminary treatment steps such as digestion or distillation. Spiked samples are analyzed at the frequency listed in **Table 1**. In this document, only ammonia-nitrogen and total phosphorus samples discussed relative to this quality assurance and quality control procedure as the other analytes discussed are not applicable to the spiking process.

When determining the amount of spike to add to a sample, the following guidelines are useful:
1. The amount of spike should be between 1 and 5 times the known expected) concentration of the sample being analyzed;

2. If the spiking procedure will result in an analytical result greater than the highest calibration standard, the amount of spike added should be modified; and

3. If the sample is expected not to contain the targeted analyte, the spike added should be at a level equal to the midrange of the calibration.

Table 7 can be used as a reference when determining the amount of spike to add to ammonia-nitrogen and total phosphorus samples.

Table 7

Preparation of Matrix Spikes for Ammonia-Nitrogen and Total Phosphorus

Analyte	Expected Sample Concentration Range (mg/L)	Stock Standard Concentration (mg/L)	Stock Standard Volume (mL) to Add	Sample + Spike Final Volume (mL)	Final Concentration (mg/L) of Spike Added
Ammonia-Nitrogen	#0.5	100	0.50	100	0.50
	>0.5 - 1.0	100	1.0	100	1.0
	>1.0 - 2.0	1000	0.20	100	2.0
	>2.0 - 5.0	1000	0.50	100	5.0
	>5.0	1000	1.0	100	10.
Total Phosphorus	#0.25	50.	0.25	50	0.25
	>0.25 - 0.50	50.	0.50	50	0.50
	>0.50 - 2.5	100	1.0	50	2.0
	>2.5 - 5.0	100	2.0	50	4.0
	>5.0 - 10.	100	5.0	50	10.

Using the examples in **Table 7**, you will observe that the volume of spike added to the ammonia-nitrogen samples never exceeds 1.0 mL. When spike preparation results in a dilution of the sample by 1% or less, the dilution is considered negligible and direct subtraction of the sample concentration from the matrix spike sample concentration is considered acceptable without a correction being made for the change in volume caused by the addition of the spike. As the final volume for ammonia-nitrogen samples is 100 mL and the maximum volume of spiked added in **Table 7** is 1.0 mL, the sample dilution does not exceed the 1% level, thus the spike recovery calculation can be performed without corrections being made for the change in volume. When the amount of spike added to the sample results in a sample dilution greater than 1%, this must be taken into account during the matrix spike percent recovery calculation - see **Equation #2** on page 29.

The addition of the added spike to a total phosphorus sample can exceed the 1.0 mL volume as total phosphorus samples are spiked prior to digestion and the final volume is adjusted to 50 mL prior to the addition of the reagents required for color development.

Note: It is extremely difficult to accurately measure spike volumes of less than 1.0 mL with a glass pipet. Measuring volumes of less than 1.0 mL can be accurately measured through the proper use of a certified Eppendorf type pipetter capable of delivering volumes less than 1.0 mL.

Matrix Spike Control Limits

Standard Methods 18th edition Table 1020:I indicates a control limit for matrix spike recoveries of 80% to 120% should be achievable in the wastewater laboratory. The associated warning limits would be 87 to 113%. For the purposes of this document, these limits are considered acceptable.

Calculating Matrix Spike Recoveries

The following **Equation #1** is used to calculate the percent recovery for matrix spike samples when the spike added results in a sample dilution of # 1%:

$$\% \text{ Recovery} = \frac{\text{observed - background}}{\text{spike amount}} \times 100$$

Where:

observed = the concentration measured in the **spiked** sample (mg/L)
background = the concentration measured in the **unspiked** sample (mg/L)
spike amount = the concentration of spike added to the sample (mg/L)

$$\text{spike amount} = \frac{\text{Volume of standard added (mL)} \times \text{Concentration of standard added (mg/L)}}{\text{Final volume of spiked sample (mL)}}$$

Example

An analyst transferred 100 mL of effluent in a beaker labeled “background”. A separate 100 mL aliquot of the same effluent sample was transferred to a separate beaker labeled “observed”. To the 100 mL effluent sample in the “observed” beaker, the analyst added 1.0 mL of a 50 mg/L ammonia-nitrogen standard as a spike. Analytical results were:

“background” beaker = 0.48 mg/L
“observed” beaker = 0.93 mg/L
spike amount = 0.50 mg/L

$$\text{spike amount} = \frac{1.0 \text{ mL} \times 50 \text{ mg/L}}{*100 \text{ mL}} = 0.50 \text{ mg/L}$$

* - as the spike added did not dilute the sample by more than 1%, the volume of the

spiked sample is not adjusted to reflect the addition of 1.0 mL of spike

Using **Equation #1** to calculate percent recovery

$$\frac{0.93 \text{ mg/L} - 0.48 \text{ mg/L}}{0.50 \text{ mg/L}} \times 100 = 90\% \text{ Recovery}$$

The following **Equation #2** is used to calculate the percent recovery for matrix spike samples when the spike added results in a sample dilution of > 1%:

$$\% \text{ Recovery} = \frac{\text{observed} - \text{background}}{\text{spike amount}} \times 100$$

Where:

observed = the concentration measured in the **spiked** sample (mg/L)
background = the concentration measured in the **unspiked** sample (mg/L)
spike amount = the concentration of spike added to the sample (mg/L)

$$\text{spike amount} = \frac{\text{Volume of standard added (mL)} \times \text{Concentration of standard added (mg/L)}}{\text{Final volume of spiked sample (mL)}}$$

Example

An analyst transferred 100 mL of effluent in a beaker labeled "background". A separate 100 mL aliquot of the same effluent sample was transferred to a separate beaker labeled "observed". To the 100 mL effluent sample in the "observed" beaker, the analyst added 5.0 mL of a 10 mg/L ammonia-nitrogen standard as a spike. Analytical results were:

"background" beaker = 0.48 mg/L
"observed" beaker = 0.93 mg/L
spike amount = 0.48 mg/L

$$\text{spike amount} = \frac{5.0 \text{ mL} \times 10. \text{ mg/L}}{*105 \text{ mL}} = 0.48 \text{ mg/L}$$

* - as the spike added diluted the sample by more than 1%, the volume of the spiked sample is adjusted to reflect the addition of 5.0 mL of spike

Using **Equation #2** to calculate percent recovery

$$\frac{0.93 \text{ mg/L} - 0.48 \text{ mg/L}}{0.48 \text{ mg/L}} \times 100 = 94\% \text{ Recovery}$$

VII. Using Control Limits

The reasons for establishing control limits in the laboratory is to help the analyst determine if

the results meet the Data Quality Objectives (DQOs) specified in the Standard Operating Procedure for each analytical method and to look for trends occurring in the laboratory which could indicate that corrective action needs to be taken prior to that specific analytical method becoming completely out-of-control, and the associated loss of defensible data. **Section VIII and Section IX** which follow discuss how the laboratory analyst should use the data from the quality control checks performed during analysis.

VIII. Evaluating Quality Control Charts

By charting the quality control checks associated with each analytical procedure, the analyst is able, through a scheduled review of these charts, to see the "big picture" of the analysis. For example, the analyst is keenly aware of the result for the glucose-glutamic acid check with the BOD_5 analysis on the day the sample is analyzed. By charting this quality control check, the analyst may observe that over a period of time there is a decreasing trend for this result. Further observation of this control chart shows this decreasing trend reverses itself each time the membrane is changed on the DO probe used for BOD_5 analysis. Because the analyst now can see the "big picture", a schedule can be implemented for the change of the DO probe membrane to prevent the BOD_5 analysis from becoming out-of-control. Another example might be: while looking at the "big picture" for the ammonia analysis matrix spike recovery, 3 consecutive results were greater than the established warning limit of 113% when the 10.0 mg/L standard was used as the spiking solution. A complete review of the ammonia-nitrogen records showed that the 10.0 mg/L standard had been prepared on the day prior to these occurrences happening. A fresh 10.0 mg/L standard was prepared and subsequent analysis using the fresh standard resulted in spike recoveries below the warning limit. Without the proper documentation, the analyst would not have known when the 10.0 mg/L standard was prepared. The point is that quality control is not only the process of analyzing blanks, replicates, matrix spikes, etc., but also keeping complete records of everything that happens in the laboratory that may affect the analysis.

IX. Corrective Action in the Laboratory

When plotting the quality control results on their respective control charts, the following are examples which indicate an analysis has reached the condition of being out-of-control and corrective action is necessary:

a. The plotted point is greater than the upper control limit or less than lower control limit

> **Corrective action = repeat the analysis immediately. If the repeat analysis is within the control limit, continue analysis; if it exceeds the control limit, discontinue analysis and correct the problem;**

b. Seven consecutive plotted points are on the same side (above or below) of the mean

> **Corrective action = analyze another sample. If the plotted point from this sample**

is on the other side of the mean, continue analysis, if not, discontinue analysis and correct the problem;

c. Five or more plotted points moving in the same direction - 5 plotted points each of which is higher (or lower) than the previous point

Corrective action = Analyze another sample. If the plotted point from this sample continues the trend of the previous plotted 5 points, discontinue analysis and correct the problem, if not, continue analysis;

d. Two of three successive plotted points are greater than the upper warning limit or less than the lower warning limit

Corrective action = analyze another sample. If the plotted point from this sample result is within the warning limit, continue analysis, if this point exceeds the same warning limit, check the method for bias (systematic error) and correct the cause prior to additional analysis.

Simply stated, corrective action is required whenever any control limit is exceeded or when definite trends are observed on the control charts. The intention of corrective action is not intended to merely create more analysis in the laboratory, but rather to establish a historical record detailing the problems that occur and how these problems were corrected. The documentation of these problems and solutions is extremely important in preventing the same problems from reoccurring in the future. Without a written record to refer to, the analysts must rely on their memory of the events that occurred. Over a period of time, important information and details may be forgotten. Additionally, any new personnel in the laboratory need to have written documentation to refer to during the "trouble shooting" process involved with corrective action.

Standard Methods outlines a step-wise procedure when performing corrective action. These steps are:

a. Check data for calculation or transcription error. Correct results if error occurred;

b. Check to see if sample(s) were prepared and analyzed according to an approved method and the laboratory's Standard Operating Procedure. If not, prepare and/or analyze again;

c. Check calibration standards against an independent standard or reference material. If calibration standards fail, prepare calibration standards again and/or recalibrate instrument and reanalyze affected samples;

d. If the Laboratory Control Sample (LCS) also known as the Laboratory Fortified Blank (LFB) fails, reanalyze another LCS;

e. If the second LCS fails, check an independent (second source) reference material.

If the second source gives an acceptable result, prepare again and reanalyze affected sample(s);

f. If a Matrix Spike sample fails, check the LCS result. If the LCS is acceptable, qualify the data for the Matrix Spike sample or analyze using another method of analysis or analyze using the method of standard additions;

g. If the Matrix Spike sample and the associated LCS sample fail, prepare again and reanalyze all affected samples;

h. If the Reagent Blank fails, analyze another Reagent Blank;

i. If the second Reagent Blank fails, prepare again and reanalyze all affected sample(s)

X. Documentation and Record Keeping

All records of equipment calibration and maintenance, quality control tests, sampling events, and laboratory analysis must be retained for a minimum of three years at the wastewater treatment facility. Before reporting any data, all raw data and calculations must be reviewed for accuracy by a person other than the person who produced the data. The person reviewing the data must be experienced enough to distinguish between correct and incorrect data.

All raw data must be kept regardless of its original form. If data is transferred to a database or some other form of record, the original data records are retained also. In summary, all samples and data must be traceable back to the analyst, date collected, time collected, date analyzed, and method of analysis used. Other required information is raw data, intermediate calculations, results and the associated reports. All quality results should be traceable to all the associated sample results.

With the many tasks facing the typical wastewater treatment personnel, documentation (the paper work) is often overlooked or not performed. In order to make data defensible, documentation is extremely important. A good saying to remember is, "if you didn't document it. . .you didn't do it". In other words, documentation means you can take credit for the work performed.

Records are to be kept in such a manner as to guarantee their permanence and security. All handwritten records are recorded in ink. The use of "erasable" ink or correction fluid of any type is not acceptable. When handwritten errors are made, the incorrect entry has a single line drawn through it and the correct entry is recorded. The initials of the person making the correction are written along with the date the correction was made.

METHODS

Determination of Biochemical Oxygen Demand (BOD_5)

BOD_5 Technique

Reference: Standard Methods, 18th edition, Procedure 5210 B, (19th and 20th edition references are also acceptable)

Apparatus:

1. 300 mL BOD bottles
2. 2-5 liter glass bottle with siphon. *Avoid using detergents to clean these bottles. Periodically clean with bleach water.*
3. 20 ± 1°C incubator
4. DO meter
5. Buret

Nutrient Solutions:

1. **Phosphate buffer:** Dissolve 8.5g KH_2PO_4(Potassium Phosphate Monobasic), 21.75 g K_2HPO_4(Potassium Phosphate Dibasic), 33.4 g $Na_2HPO_4 \bullet 7H_2O$(Sodium Phosphate Dibasic Heptahydrate), and 1.7 g NH_4Cl(Ammonium Chloride) in approx. 500 mL reagent water. Dilute to 1 L. The pH should be 7.2. Store in 4°C refrigerator. Check before each use for contamination (if there is any indication of biological/microbial growth, discard remaining reagent and prepare fresh).
2. **Magnesium sulfate solution:** Dissolve 22.5 g $MgSO_4 \bullet 7H_2O$ in reagent water. Dilute to 1L.
3. **Calcium chloride solution:** Dissolve 27.5 g $CaCl_2$ in reagent water. Dilute to 1L.
4. **Ferric Chloride solution:** Dissolve 0.25 g $FeCl_3 \bullet 6H_2O$ in reagent water. Dilute 1 L.

Preparation of Dilution Water:

1. Dilution water is prepared by adding one mL of each nutrient solution per liter of reagent water. Dilution water should be allowed to equilibrate in the incubator or in the lab for at least 24 hours at room temperature (68-72°F or 20-22°C).

2. It is important that dilution water be saturated with oxygen. Saturation is accomplished by bubbling filtered, compressed air into the dilution water.

Pretreatment of Sample:

1. **The diluted sample used to determine BOD_5 must have a pH between 6.5 and 7.5**. For municipal sewage or effluent, the pH range is generally between 5-9, but the buffering capacity of the phosphate buffer will often bring the pH of the dilution that uses the most sample to confirm that the dilutions lie in the proper pH range. As needed neutralize samples with 1N sulfuric acid or 1N sodium hydroxide (base). Do not dilute the sample with the acid or base by more than 0.5% (1.5 mL in a 300 mL BOD bottle).

2. If any type of chlorination process is employed during treatment, final effluent samples are initially tested for the presence of residual chorine. Dechlorination is required if a chlorine residual is present. Residual chlorine can kill the microorganisms that are critical to BOD_5 analysis. See pages 39 or 40.

3. Samples supersaturated with dissolved oxygen, over 9.0 mg/L at 20° C, may be encountered during winter months in localities where algae is actively growing (lagoons). To prevent loss of oxygen during incubation of these samples, the DO should be reduced by shaking the sample or aerating it with filtered compressed air. *These types of samples often have a high concentration of nitrifying organisms, that can lead to bias in BOD_5 results.*

4. Samples of industrial wastes, disinfected wastes, high temperature wastes, or wastes with extreme pH values may not contain enough microorganisms to oxidize the biodegradable matter in the samples. Such samples must be seeded. See BOD_5 Seeding Procedure on page 42.

5. If carbonaceous BOD_5 is required in your NPDES permit, it is necessary to add nitrification inhibitor to all samples (including blanks, GGA and seed controls).

Dilution Technique:

1. Estimate the BOD_5 of the sample and select suitable dilutions from the following tables:

Estimated BOD_5 (mg/L)	Suggested Sample Volumes (mL)
< 5	200, 250, 300
<10	100, 150, 200
10-30	25, 50, 100
60-90	10, 15, 25

Estimated BOD_5 (mg/L)	Suggested Sample Volumes (mL)
90-150	5, 10, 15
150-300	1, 3, 5
300-700	0.5, 1.3
1500-2500	0.25,0.5,1

When preparing replicate samples for quality control purposes, prepare the replicate at exactly the same dilutions as the original sample.

2. Using a **large-tipped** pipet for samples less than 50 mL or a graduated cylinder for larger sample volumes, measure the proper amount of well-mixed sample into thoroughly cleaned and rinsed 300 mL bottles. ******Dilutions less than 3 mL must be made by diluting the sample in a Class A graduated cylinder or a Class A volumetric flask before pipetting the sample volume used in the BOD bottle.******

3. Each BOD bottle is filled slowly, preventing the introduction of air bubbles, adding dilution water so that the stopper can be inserted without leaving an air bubble. The siphon hose must be made of surgical gum (latex rubber), polypropylene or polyethylene. (NO OTHER MATERIALS MAY BE USED)

4. Completely fill at least two bottles with dilution water to be incubated as blanks.

5. The benchsheet on page 78 indicates the information that needs to be recorded when analyzing BOD_5.

Dissolved Oxygen (DO) and BOD_5 Determinations:

1. With each use of the DO probe, check the membrane for:
   - ✓ Wrinkles
   - ✓ Discolorations
   - ✓ Bubbles
   - ✓ Suspended matter in filling solution
   - ✓ Surface slime

   If any of the above are observed, replace the membrane per specific manufacturer's instructions.

2. Read and record the barometric pressure each day of analysis. This can be from a barometer in the laboratory. Barometric pressure readings should not be corrected to sea level.

3. Calibrate the instrument used for BOD_5 analysis each day of use.

4. Determine the initial DO of all bottles in the analytical run and record on benchsheet.

5. Fill water seals with dilution water and snap on plastic caps to reduce evaporation from seals. Place all bottles in the analytical run in a $20 \pm 1^{o}C$ incubator for 5 days. Check daily, add water to seals if necessary.

6. After removing from the incubator and before removing the stoppers, pour off excess water.

7. After 5 days determine the DO of all samples in the analytical run and record the result as Final DO.

Calculations:

For unseeded samples, use the following formula:

$$\mathbf{BOD_5\ mg/l = (Initial\ DO - Final\ DO) \times Dilution\ Factor}$$

$$\mathbf{Dilution\ Factor = \frac{Bottle\ Volume\ (300\ mL)}{Sample\ Volume}}$$

For seeded samples, use the following formula:

$$\mathbf{BOD_5\ mg/L = (Initial\ DO - Final\ DO) - seed\ correction \times dilution\ factor}$$

$$\mathbf{Dilution\ factor = \frac{Bottle\ Volume\ (300\ mL)}{Sample\ Volume}}$$

Notes:

1. Standard Methods recommends that the dilution water blank depletion not exceed 0.2 mg/L, if the DO depletion in the dilution water blanks exceeds 0.2mg/L the results of the test are questionable. Do not subtract blank values from sample results.

2. Only dilutions with DO depletions of at least 2 mg/L, and final DO of at least 1 mg/L may be used to calculate BOD.

3. If there is no evidence of toxic effects, average the results from all dilutions that meet the minimum oxygen depletion and minimum residual DO requirements.

4. A toxic effect is indicated when BOD increases significantly as the sample dilution increases. This is often referred to as “sliding BODs”.

Standardization of DO meter- Winkler Titration Technique

Reference: Standard Methods, 18th edition, Procedure 4500-O C (19th and 20th edition references are also acceptable)

Reagents:

1. **Manganous sulfate solution**: Dissolve 480 g $MnSO_4 \bullet 4H_2O$ in reagent water. Filter; dilute to 1 L.
2. **Alkali-iodide-azide reagent**: Dissolve 500 g NaOH(Sodium Hydroxide) and 135 g NaI(Sodium Iodide) in reagent water. Dilute to 1 L. Add 10 g NaN_3(Sodium Azide) dissolve in 40 mL reagent water. This reagent should not give a color with starch solution when diluted and acidified.
3. **Concentrated Sulfuric acid(H_2SO_4)**
4. **Standard sodium thiosulfate titrant, 0.0250N:** Purchase commercially.
5. **Starch Solution:** prepare an emulsion of 5 g soluble starch in a beaker with a small amount of distilled water. Pour this emulsion into 1 L of boiling water, allow to boil a few minutes, and let settle overnight. Use the clear supernate. This solution may be preserved by the addition of 1.25 g salicylic acid/L and storage at 4°C.

Procedure:

1. Slowly siphon three portions of aerated dilution water into three separate BOD bottles. Avoid adding atmospheric O_2 to dilution water.

2. To two of the three BOD bottles, add 1 mL $MnSO_4$ solution, followed by 1 mL alkali-iodide-azide reagent. Submerge pipette tips in sample when adding reagents. Rinse tips well between uses.

3. Stopper carefully to exclude air bubbles; mix by inverting bottle several times.

4. When precipitate has settled to about half the bottle volume, carefully remove the stopper and 1.0 mL conc. sulfuric acid. Re-stopper and mix by gentle inversion until the iodine is uniformly distributed throughout the bottle.

5. Transfer 201 mL of sample into a 250 mL titration flask and titrate with 0.0250N Sodium thiosulfate to a pale straw color. Add 1-2 mL of starch solution and continue to titrate to first disappearance of the blue color. .(200 mL of original dilution water is equal to 201 mL of dilution water plus reagents.)

6. Titrate two of the three samples. Results should be within 0.1 mL if using a buret with increments of 0.05 mL. Calibrate the DO probe with the third bottle.

Pretreatment of Chlorinated BOD Samples

Reagents:

1. **Acetic acid solution, 1+1**: Carefully add 500 mL. Acetic acid $C_2H_3O_2$, concentrated (glacial) to 500 mL of reagent water.

2. **Potassium Iodide Solution**: Dissolve 10 grams Potassium Iodide, KI, in a 100 mL volumetric flask. Bring to volume with reagent water.

3. **Sodium Sulfite Solution, 0.0250N**: Dissolve 1.575 grams anhydrous Sodium sulfite, Na_2SO_3, in a 1,000 mL volumetric flask. Dilute to volume with reagent water.

   Note: This solution is not stable and must be prepared daily.

4. **Starch Indicator Solution (For Analysis with Iodine):** Prepare an emulsion of 5 g soluble starch in a mortar of beaker with a small amount of reagent water. Pour this emulsion into 1 L of boiling water, stir, and let settle overnight. Use the clear supernate. This solution may be preserved by the addition of 1.25 g salicylic acid /L and stored at 4^oC.

Procedure:

1. Conduct a chlorine residual analysis on a portion of the sample collected. *Potassium iodide/starch paper can be used as a quick qualitative tests for residual chlorine*. If no residual is found, proceed with the BOD analysis utilizing seeded dilution water. If a residual is found, proceed with the following steps before initiating the BOD test.

2. Determination of volume of Sodium Sulfite needed to neutralize chlorine residual in sample prior to BOD_5 analysis.

   a. Obtain a 200 mL portion of the sample to be tested.
   b. Add 10 mL of 1+1 acetic acid solution
   c. Add 10 mL of potassium iodine solution
   d. Add 2 mL starch
   e. Titrate with 0.025N sodium sulfite. The end point has been reached when a clear color persists after complete mixing.
   f. Measure volume of 0.025N sodium sulfite used.

3. Sample Pretreatment

   a. Obtain a separate 200 mL portion of the same sample used in Step 2.
   b. Add to this sample the same volume of 0.025N sodium sulfite solution that was determined in Step 2.e and mix.
   c. Retest for residual chlorine after allowing the sample to stand for 10-20 minutes. If no residual chlorine is present, proceed with the BOD analysis. Samples that have been chlorinated must be seeded.

Procedure for Dechlorinating Final Effluent BOD Samples *(quick method)*

Residual chlorine may dissipate in chlorinated samples if the sample is allowed to stand for one to two hours prior to BOD analysis. For those samples where this does not occur, chlorine residual is destroyed by the addition of a 10% Sodium thiosulfate $Na_2S_2O_3$ solution.

Reagents and equipment:

DPD Powder Pop Dispenser or DPD Powder pillows for chlorine for use with 10 mL of sample

Sodium thiosulfate ($Na_2S_2O_3$) 10% solution: Prepare by dissolving 10 g Sodium thiosulfate in 50 mL reagent water. Dilute to volume in a 100 mL Class A volumetric flask. Store in a refrigerator. Prepare fresh weekly.

Procedure:

1. Transfer 10 mL chlorinated sample to a clean test tube. Add the contents of one DPD Powder pillow or one shot of reagents from the DPD Powder Pop Dispenser. If a pink color develops, chlorine residual is present in the sample.

2. Add one drop of 10% Sodium thiosulfate to the sample to designated for BOD analysis and mix thoroughly.

3. Check the sample in #2 using the procedure in #1.

4. If no pink color develops, the sample is ready for BOD analysis if all other sample pretreatment procedures have been performed.

5. If a pink color develops, repeat steps #2 and #3 until no pink color is observed.

As Sodium thiosulfate is very effective in neutralizing the chlorine, usually one drop is sufficient to remove chlorine from chlorinated samples.

Use the Sodium thiosulfate with caution as it has an oxygen demand and excess amounts will result in elevated BOD results.

After the Final Effluent sample is dechlorinated, it must be seeded during the BOD sample setup procedure. Use the BOD seeding procedure found in this document.

Preparation of Glucose-Glutamic Acid Standard (GGA)

Reagents:

Note: The Glucose/Glutamic acid solution can be purchased commercially. If purchasing this standard, check to make sure that each Liter of standard contains 150 mg of Glucose and 150 mg of Glutamic acid.

1. Reagent grade Glucose, , $C_6H_{12}O_6$

2. Glutamic acid, $HOCOCH_2CH_2CH(NH_2)COOH$

Procedure:

1. Dry reagent grade Glucose and Glutamic acid at 103°C for 1 hour. Cool for one hour in the desiccator.

2. Dissolve 150 mg (0.150 g) of Glucose and 150 mg (0.150 g) Glutamic acid in reagent water and dilute to 1000 mL with reagent water in a Class A volumetric flask.

   Note: This solution will become contaminated quickly and must be used immediately unless the following is done:

   Place into each of several milk dilution bottles or test tubes the quantity of GGA standard that will be used each time this standard is analyzed.

   Seal. Place caps on the bottles or test tubes loosely (about a quarter turn) so they can't readily fall off but can still vent steam and sterilize for thirty minutes. Use caution, if the caps are too tight the vessels WILL explode. These sterilized portions can then be cooled to room temperature, then TIGHTEN the caps and store at 4°C. These sterilized standards can be used for up six months if not opened.

   When analyzing this standard, pipet six mL of GGA standard into each BOD_5 bottle being used for analysis of the GGA standard. This is critical! The BOD_5 concentration of 198± 30.5 mg/L for this standard is based on a 2% dilution of GGA (6mL/300 ml BOD bottle). It is important not to use dilutions other than the 2% dilution.

   Seed is then added and the bottles filled with BOD dilution water. These bottles are incubated and BOD is determined in the same manner as other BOD samples.

The acceptable BOD_5 value of the standard is 198± 30.5 mg/l. If the calculated result falls outside this range, the cause of the problem must be identified. Treatment plant sample results obtained using the same seed and dilution water as were used in analyzing the GGA standard may need to be qualified. Once the problem has been identified and corrected through additional analyis of the GGA standard, you should document what caused the problem for future reference

BOD Seeding Procedure

Before planning to seed samples, conduct a study to determine the amount of seed to add to seed controls and samples. Instructions for the study:

Preparation of Seed:

1. Collect a raw influent grab sample the day before performing the test. If the influent contains significant industrial loading, settled mixed liquor may provide a better seed than raw influent. If used for seed, settled mixed liquor does not need to be incubated at 20 °C overnight. Seed can also be commercially obtained. There are at least two products widely used: BioSeed ™, and PolySeed ™. NOTE: Raw influent grab sample should be taken at the same time of day each time seeding material is needed. This will help ensure that samples are somewhat uniform.

2. Place raw influent grab sample in incubator (20°C) overnight.

Preparation of Seed Controls – Initial Study

1. Take the incubated raw influent sample out of the incubator -- DO NOT MIX.

2. Pipet 3, 6, 9, 12, 15, and 18 mL of the clear supernatant into six BOD bottles respectively.

3. Fill these six bottles with BOD dilution water.

4. Determine the initial dissolved oxygen ($DO_{initial}$) on each of the six bottles.

Calculation of Seed Correction-Initial Study

1. After the 5 day incubation, determine the final dissolved oxygen (DO_{final}) on each of the six seed controls set up in the section above.

2. Ideally, one of the six seed controls will have close to 50% dissolved oxygen depletion. If this 50% dissolved oxygen depletion is not obtained, repeat the **Initial Study** using larger volumes of the clear supernatant until the 50% dissolved oxygen is obtained. NOTE: each study will require that you start with a new raw influent grab sample that has been incubated and allowed to settle overnight.

3. For each seed control dilution analyzed, calculate the DO lost per mL.of seed used as follows:

$$\frac{DO_{initial} - DO_{final}}{\text{mL raw influent supernatant used}}$$

Example: 9 mL of incubated raw influent supernatant was added to a 300 mL BOD bottle and the bottle was then filled with BOD dilution water. The $DO_{initial}$ = 8.8 mg/L. After the 5 day incubation period, the DO_{final} = 4.3 mg/L. Using the formula above:

$$\frac{8.8 \text{ mg/L} - 4.3 \text{ mg/L}}{9 \text{ mL seed added}} = \frac{4.5 \text{ mg/L}}{9\text{mL}} = 0.5 \text{ mg/L DO lost per mL of seed added}$$

4. Use the same rule for DO depletion criteria as in all other BODs (at least 2.0 mg/L DO depletion and at least 1.0 mg/L residual DO (after 5 days) (*Standard Methods*, 18th Edition).

5. If more than one of the seed controls meets the DO depletion criteria, referred to in #4, calculate the <u>average</u> DO lost per mL of seed (See Table 1).

Table 1

Initial Study to Determine how many milliliters of Incubated Raw Influent Supernatant (Seed) to Use in Seed Controls

Bottle #	Seed Added (mL)	$DO_{initial}$ (mg/L)	DO_{final} (mg/L)	DO Lost (mg/L)	DO Lost per mL of Seed
A	3	8.9	7.4	1.5	***
B	6	8.8	5.9	2.9	0.48
C	9	8.8	4.2	4.6	0.51
D	12	8.7	2.8	5.9	0.49
E	15	8.8	1.4	7.4	0.49
F	18	8.8	0.2	8.6	***

*** = Did not meet the criteria of 2.0 mg/L DO loss or 1.0 mg/L DO residual

In Table 1 above, it is observed that the sample with 9 mL of Seed Added lost approximately 50% of the $DO_{initial}$, thus by setting up Seed Controls with 6, 9, and 12 mL respectively, we can be fairly confident that at least one of the Seed Controls will give you a DO depletion that meets the criteria referenced in #4.

Using Table 1, the <u>average</u> DO lost per mL of Seed Added =

$$\frac{0.48 + 0.51 + 0.49 + 0.49}{\text{Number of valid results}} = \frac{1.97}{} = 0.49 \text{ DO lost/mL of Seed Added}$$

<u>This 0.49 DO lost/mL of Seed, or rounded off, 0.5 is now referred to as the SEED CORRECTION</u>

Calculating Amount of Seed to Add to the Effluent Sample – Initial Study

If the seed correction falls in the range of 0.6 – 1.0 per milliliter of seed, it should be sufficient to add 1 mL. of seed to each of your BOD bottles when you are conducting your usual tests. If the seed correction falls in a range below 0.6 and the seed controls met the DO depletion criteria, the amount of seed added to each of your BOD bottles will need to be such that the number of mL added multiplied by the seed correction falls within the range of 0.6 to 1.0.

For example: using the seed correction figured in **Table 1** above of 0.5, if 2 mL are added to BOD samples

$0.5 \times 2 = 1.0$

if seed correction is 0.3, if 2 mL are added to BOD samples

$0.3 \times 2 = 0.6$

if seed correction is 0.7, should one mL is sufficient to add to BOD samples

Now you should have a reasonable idea of what volumes of seed will be needed to add to your final effluent BOD samples to meet the depletion criteria and how to figure the seed correction number that will be subtracted from the BOD_5 calculation.

Seeding Procedure for your <u>Daily</u> Final Effluent BOD testing

Preparation of Seed

Follow steps 1 and 2 in Initial Study under Preparation of Seed.

Preparation of Seed Controls

Using calculations determined in your Initial Study that include the volumes that gave approximately 50% depletion, follow steps 1 through 4 in the Initial Study instructions under Preparation of Seed Controls. NOTE you will only be using 3 dilutions not 6 as in the Initial Study.

Preparation of Seeded BOD Samples

1. Fill the bottles approximately $^1/_3$ to ½ with dilution water.

2. Pipet amount of seed (supernatant) that you have already determined will give the needed depletion into each of your final effluent BOD sample bottles.

3. Add the appropriate amount of sample (final effluent) to each of the bottles.

4. Complete the filling of the BOD bottles with dilution water.

5. Determine the initial dissolved oxygen ($DO_{initial}$)on each of the bottles.

Calculation of Seed Correction

1. After the 5 day incubation, determine the final dissolved oxygen (DO_{final}) on each of the three seed controls set up in the section above (and the final dissolved oxygen (DO_{final}) on the rest of your BOD samples as is normal).

2. Follow steps 3, 4, and 5 in the Initial Study under Calculation of Seed Correction.

Calculation of BOD_5 in sample

$$BOD_5 = \text{BOD mg/L} = (BOD_{initial} - BOD_{final}) - \text{seed correction x dilution factor}$$

$$\text{Dilution factor} = \frac{300}{\text{Sample size (mL)}}$$

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Determination of Total Suspended Solids

1. SCOPE AND APPLICATION

1.1. Total Suspended Solids (TSS) also referred to as Non-Filterable Residue is defined as the amount of solids in a sample that are larger than 2 microns. This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes. The practical range of determination is 4 mg/L to 20,000 mg/L. **This method is based on Standard Methods, 18th edition, Procedure 2540 D. Referencing Standard Methods 19th, 20th, and EPA Method 160.2 are also acceptable.**

2. SUMMARY OF METHOD

2.1. A well-mixed sample is filtered through a pre-weighed glass fiber filter. The filter and any residue are then dried to a constant weight at 103 – 105^{o} C. The filter is cooled in a desiccator, weighed and the results used to compute the TSS of the sample.

Note: If the filtrate of this analysis is collected in a clean filtering flask, it may be used for a Total Dissolved Solids (TDS) determination if the sample requires both tests.

3. APPARATUS

3.1. Glass fiber filters (without organic binder), 934 AH, or equivalent e.g. Whatman GF/C
3.2. Filter Holder or Gooch crucibles or Buchner funnel
3.3. Aluminum weigh dishes (not needed if using Gooch crucibles or Buchner funnel)
3.4. Membrane Filter Funnel (not needed if using Gooch crucibles or Buchner funnel)
3.5. Filtering Flask
3.6. Filter Support: Filtering apparatus with reservoir and a coarse fritted disc as a filter support (not needed if using Gooch crucibles or Buchner funnels)
3.7. Drying Oven with an adjustable temperature control knob capable of heating up to 105^{o} C
3.8. Desiccator
3.9. Analytical Balance capable of weighing to 0.0001 g (0.1 mg)
3.10. Forceps and/or tongs
3.11. Aluminum foil
3.12. Graduated Cylinders (assorted sizes)
3.13. Wide Bore Pipets (Blowout type)

4. REAGENTS AND CHEMICALS

4.1. Reagent Water
4.2. Desiccant – a mixture a blue (97% $CaSO_4$ and 3% $CoCl_2$ – size 8 mesh) and white (100% $CaSO_4$ – size 10-20 mesh) desiccant or a commercially available self contained desiccant package
4.2.1. A mixture of approximately 10% blue and 90% white desiccant should be placed in the desiccator used to store filters, crucibles, and aluminum weigh dishes
4.2.2. When the desiccant turns from blue to pink (indicating water absorption), heat the desiccant in an 180^{o} C oven for a minimum of one hour. After cooling the desiccant, it is ready to be reused. Reuse the desiccant three times. After three times, discard the old desiccant and replace with new desiccant. The old desiccant may be disposed with the normal laboratory waste. If using a commercially available self contained desiccant package, consult the manufacturer's instructions for the correct heating temperature. Commercially packaged desiccants usually involve the use of plastics that may require a lower heating temperature and a long drying time.

5. SAMPLE HANDLING AND PRESERVATION

5.1. When analysis cannot be performed within two hours of sample collection, preservation by refrigeration or icing to 4^{o} C is required to minimize the microbiological decomposition of solids. Analysis should begin as soon as possible after collection.
5.2. Non-representative solids such as grit, seeds, leaves, sticks, and lumps of fecal matter should be removed from

the sample prior to analysis if the goal of the analysis is not to include non-representative solids in the final result. Floating oil and grease, if present, should be included in the sample and dispersed by vigorous mixing before measuring the sample aliquot to be analyzed.

5.3. Samples stored at 4° C should be brought to room temperature before processing.

5.4. Sample must be analyzed within seven days of collection.

6. SAMPLE PREPARATION AND HANDLING PROCEDURES

Note: Be sure to record all the necessary information neatly and accurately on the TSS bench sheet.

6.1. Preparation of the glass fiber filters

6.1.1. Place each glass fiber filter on the membrane filter apparatus rough side up (if using Gooch crucibles or Buchner funnels, place glass fiber filter in a clean Gooch crucible or Buchner funnel). While applying a vacuum, wash each filter with three successive 20 mL volumes of reagent water allowing the water to be completely pulled through the filter between washings.

6.1.2. Using filter forceps (use tongs when handling Gooch crucibles), remove the filter from the filtering apparatus, place on clean aluminum foil and dry in the oven at 103 – 105° C for a minimum of one hour (Gooch crucibles do not need placed on clean aluminum foil). The thermometer used to check the temperature of the oven must have a Certificate of Traceability to a NIST certified thermometer or the thermometer must be compared to a NIST traceable thermometer on an annual schedule with any correction factor recorded and included with the thermometer. After heating at 103 – 105° C for a minimum of one hour, cool the filters and/or Gooch crucibles containing filters in a desiccator for at least 30 minutes prior to weighing.

6.1.3. After verifying the calibration of the analytical balance, weigh the filters and/or Gooch crucibles and record the weight. Store in the desiccator until ready to use.

6.2. When ready to filter a sample, assemble the filtering apparatus: Connect the vacuum hose to the filter flask. Place the filter support (or Gooch crucible holder or Buchner funnel) on the filter flask. Put the filter, rough side up (or Gooch crucible or Buchner funnel) on the filtering apparatus. If using a filter, place the membrane filter funnel on top of the filter. Begin vacuum suction.

6.3. While the filtering apparatus is operating, shake the sample vigorously and transfer an aliquot of the well-mixed sample to the filtering apparatus using a clean graduated cylinder or wide bore pipet. The volume of sample filtered should be such that it gives a solids weight of at least 0.0010 g (1.0 mg) but not more than 0.2 g (200 mg). Record volume of sample used on TSS bench sheet. For sample volumes less than 25 mL, a wide bore pipet is recommended for measuring the volume of the sample to be filtered. **Note:** If TDS is also going to be determined, be sure to remove the filtrate from the filtering flask prior to rinsing the graduated cylinder or pipet, filter, and sides of the filtering device. This will ensure the filtrate used for TDS is not diluted. After all the liquid has filtered through, wash the graduated cylinder or pipet, filter, and sides of the filtering device with three 10 mL portions of reagent water. Continue to apply the vacuum for three minutes after the sample has completely passed through and the filter has been rinsed, to assure that all traces of water have been removed.

6.4. Carefully remove the filter with forceps (remove Gooch crucibles with tongs) and place filter into aluminum weigh dish labeled with the sample location. Place aluminum weigh dish and/or Gooch crucible in an oven set at 103 – 105° C. Wash the graduated cylinder or pipet, the filter holder, and membrane filter funnel then rinse with reagent water before filtering the next sample to avoid sample carryover. **Note:** If also analyzing for TDS, the filter flask must also be washed and rinsed with reagent water. Dry the sample filters and/or Gooch crucibles for a minimum of one hour (overnight drying ensures thorough drying).

6.5. Remove the dried aluminum weigh dishes containing filters and/or Gooch crucibles and place in desiccator to cool for at least 30 minutes. Check the calibration of the balance before proceeding. Weigh the filters and/or Gooch crucibles and record weight on TSS bench sheet. Do not leave the desiccator open too long. If the filters or crucibles are exposed to the laboratory air for a long period of time, the sample results may be affected.

6.6. On a routine basis, repeat the drying cycle in the 103 – 105^{o} C oven for one more hour, then cool in the desiccator and weigh again using the same procedures described in step 6.5. The weight obtained in step 6.6. should agree within 4% or 0.0005 g (0.5 mg) of the weight recorded in step 6.5. If it does not, longer drying times may be required in step 6.4. to ensure complete drying of the samples.

6.7. Determination of Total Suspended Solids
The Total Suspended Solids is calculated using the following equation:

$$TSS(mg/L) = \frac{(\text{weight of dry filter + residue (g)} - \text{weight of dry filter (g)}) \times 1{,}000{,}000}{\text{sample volume (ml)}}$$

7. QUALITY CONTROL

7.1. One blank per batch of 20 samples or less. The result for the blank must be below the current Level of Detection (LOD) in order to report the results for the batch of samples without qualification. If the result for the blank is greater than the LOD, the blank and all associated samples may be rerun until the blank passes.

7.2. One set of sample duplicates per batch of 20 samples or less. The calculated precision value (RPD) must meet the control limit guidelines outlined earlier in the Quality Assurance Manual. If the duplicates exceed the acceptable control limit, they must be rerun until an acceptable RPD is achieved.

7.3. A Laboratory/Quality Control Standard must be analyzed on a quarterly basis. The laboratory results for this standard should fall within the acceptance limits accompanying the standard. An acceptable alternative to this is to split samples with another laboratory. The RPD for split samples should be less than or equal to 20% to be acceptable.

8. SAFETY/HAZARDOUS WASTE MANAGEMENT

8.1. Safety glasses, gloves, and lab coats should be worn at all times while processing samples.

8.2. Excess samples may be flushed down the sink unless they are determined to be hazardous waste.

9. REFERENCES

9.1. Standard Methods 18th edition, 2540 D.

9.2. Quality Assurance Manual

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Determination of Ammonia

Ion Selective Electrode (ISE) technique

References: Standard Methods, 18th edition, Procedure 4500-NH3 F (19th and 20th edition references are also acceptable)
Manufacturer's manual

I. INTRODUCTION

The ammonia selective electrode uses a hydro-phobic gas permeable membrane to separate the sample solution from an electrode internal solution of ammonium chloride.

Dissolved ammonia (NH_3 (aq.) and NH_4) is converted by raising the pH above 11 with a strong base. NH_3 (aq.) diffuses through the membrane and changes the internal solution pH that is sensed by a pH electrode. The fixed level of chloride in the internal solution is sensed by a chloride ion-selective electrode that serves as the references electrode. Potentiometric measurements are made with a pH meter having an expanded millivolt scale or with a specific ion meter.

The ion selective electrode method for NH_3 is applicable to the measurement of 0.03 to 1400 mg NH_3-N/L in domestic and industrial wastes (Ref. Std. Methods, 18th ed., pg 4-81). High concentrations of dissolved ions affect the measurement, but color and turbidity do not. Sample distillation is unnecessary in the absence of interferences. Use standard solutions and samples that have the same temperature and contain about the same total level of dissolved species. The ammonia-selective electrode responds slowly below 1 mg NH_3-N/L; hence use longer times of electrode immersion (5 to 10 min.) to obtain stable readings.

II. SAMPLING, STORAGE AND PRESERVATION

Ammonia samples should be preserved with 1:1 H_2SO_4 to a pH of less than 2 and stored at 4°C. Samples to be measured can be either grab or composite whichever is required in the NPDES permit. The samples must be preserved as they are collected to prevent escape of NH_3 gas. Use 1 mL of 1:1 $H_2SO_4$to 500 mL of sample. Samples can be checked with pH paper to ensure preservation is adequate. Preserved samples can be held for analysis up to 28 days at 4°C.

III. APPARATUS

A. Reagents

1. Sulfuric Acid 1:1, add a volume of concentrated H_2S0_4 to an equal volume of deionized water. Mix well in a beaker or flask immersed in cold water. DO NOT ATTEMPT TO PREPARE THIS SOLUTION IN THE ACID BOTTLE. Allow to cool at room temperature. This solution is used to preserve samples.
   NOTE: Always add acid to water! A highly exothermic reaction takes place when making this dilute acid. DO NOT place into cold water once the flask is hot!. Prepare a cold waterbath to constantly cool the mixture.

2. Sodium Hydroxide (NaOH) 10 N, dissolve 400g NaOH in 800 mL of Milli-Q water, cool and dilute to 1000 mL or use Orion ammonia pH adjusting ISA (5M NaOH, 10% Methanol, color indicator, 0.05 M Disodium EDTA). Orion Cat. No. 951211.

3. Fresh Milli-Q water for making all dilutions and blanks. Should be NH_3 free.

4. Electrode internal filling solution Orion Cat. No. 951202.

5. 1000 ppm Ammonia Standard Orion No. 951007.

B. Equipment

- 150 mL glass beakers
- 100 mL, 1 L, 2 L volumetric flasks: Class A
- 1 mL, 3 mL, 5 mL, 10 mL volumetric pipettes: Class A
- Teflon coated stirring bars
- thermally insulated magnetic stirrer
- ammonia electrode Orion model 95-12
- 1 mL, 5 mL, 10 mL, 25 mL graduated pipettes
- Orion pH/ISE meter model 920A or its equivalent

IV. PROCEDURE

A. Preparation of Standards

1. Prepare 1 L of 100 ppm standard from the 1000 ppm stock solution (100 mL of 1000 ppm stock ÷ 900 mL of D-l H_20). 100 ppm standard stores for up to 6 months.

2. Prepare 2 L of 10 ppm standard from the 100 ppm standard (200 mL of 100 ppm ÷ 1800 mL of D-l H_20). Stores up to 2 weeks.

3. Prepare 2 L of 3 ppm standard from the 10 ppm standard (600 mL of 10 ppm + 1400 mL of D-l H_20). Stores up to 2 weeks.

4. Prepare 1 L of 1 ppm standard from the 10 ppm standard (100 mL of 10 ppm + 900 mL of D-l H_20). Stores up to 2 weeks.

5. Prepare 100 mL of 0.1 ppm standard from the 1 ppm standard (10 mL of 1 ppm + 90 mL D-l H_20). Make fresh each time meter is calibrated.

B. Meter Calibration (Orion 920A)

1. Pressing any key when it is in stand-by mode turns on the meter. Immerse the probe in Milli-Q water for 1 to 2 minutes while stirring. Do not stir so rapidly that a vortex is created.

2. Creating a vortex may introduce air bubbles into the solution. If they become trapped on the electrode membrane they will cause interference. Stir just fast enough to mix the solution well. Maintain the same stirring rate throughout the calibration and testing procedures.

3. Standards and samples should be at room temperature during testing.

4. To calibrate the meter, start by pressing the "Calibrate key". The meter will display the date and time of last calibration.

5. The display asks for the number of standards to be used in the calibration. Press the number "3" key and then press the "yes" button.

6. Now proceed with the introduction of the standards. Calibrate starting with 100 mL of the lowest standard (0.1 ppm).

7. Add a sufficient volume of 5 M NaOH (usually 1 mL is sufficient) to raise the pH above 11. If using Orion ISA solution, the sample will turn blue when the pH is 11 or greater. Do not add NaOH solution before immersing electrode since ammonia may be lost from the basic solution. If the electrode is not immersed when this happens it cannot detect the ammonia that is released during this time.

8. Keep the electrode in solution until a stable reading is obtained.

9. After the standard stabilizes and a ready message comes on the screen, key in the standard's concentration value and then press the "yes" key so the meter will accept the value.

10. Check and record the millivolt reading by pressing the "2^{nd}" button and then the "mv" key**.** Return to the concentration mode after recording my by pressing the measure key.

11. Repeat the steps from above for the remaining standards, proceeding from lowest to highest in concentration. Note:If the electrode is functioning properly a tenfold change of NH_3 -N concentration produces a mv potential change of 59 my.

12. After the calibration is complete, check the curve with a 3 ppm ammonia standard. This standard, for Q.C. purposes, should fall between 95% to 105% recovery (2.85 ppm — 3.15 ppm).

13. A blank sample should also be run. It is important to show that your fresh Milli-Q water measures less than 0.1 ppm ammonia, which is your lowest standard value and detection limit.

14. If all of the above checks out, proceed with analysis of samples.

15. All sample volumes are based on a 100 mL final volume. Record sample concentration values from a stable meter display. Dilute samples if necessary to bring the ammonia concentration to within the calibration curve range (0.1 ppm to 10 ppm).

16. If an aliquot of sample needs to be diluted to 100 mL, a dilution factor will need to be

used to calculate the original sample concentration. Always make sure you rinse the probe between samples to prevent carryover.

17. Periodically run a 3 ppm ammonia standard to check the slope of the calibration curve over time.

18. When sample analysis is complete, shut down the meter by giving the probe a final rinse, immersing it in 1 ppm ammonia standard for short term storage and pressing the Standby button on the meter.

V. QUALITY CONTROL

A. A 3 ppm standard should be run each time the meter is calibrated. The value obtained must fall between 95% - 105% of the true value of 3 ppm. This standard should also be run at the end of the test.

B. A duplicate sample is analyzed with each analytical run on the raw and the final. Run duplicates on final samples only if its concentration falls above 0.1 ppm ammonia.

C. Spiked samples should be run on both final and raw samples. Once again only run a final spike if its concentration falls high enough above the detection limit. As a general rule of thumb, the spiked sample should run about twice the concentration of the original sample. All measured duplicates and spikes should fall between posted control limits.

D. Approved Reference Standards are analyzed at least quarterly and

E. Results fall within the 95% Confidence Interval and/or split samples are analyzed at least quarterly and results fall within 20% RPD.

F. A 3 ppm standard of a different Lot number or source should be run each time the meter is calibrated. The value obtained must fall between 95% - 105% of the true value of 3 ppm. This standard should also be run at the end of the test.

VI. SPIKE CALCULATIONS

A. Use this formula for sample aliquots less than 100 mL that are then diluted to a final volume of 100 mL. These types of samples are usually RAW influent samples.

% Recovery of spike =

$$\frac{(\text{meter reading spiked sample}) - (\text{meter reading unspiked sample}) \times 100}{\text{(calculated concentration of spike in 100 mL)}}$$

B. Use these formulas for samples measured with no dilution, which is 100mL of sample. This type of sample usually falls below 10 ppm without dilution, such as final effluent.

1. True concentration of spike =

$$\frac{(\text{standard conc}) \times (\text{volume of standard used for spike})}{(\text{volume of the spike} + \text{volume of the sample})}$$

example: $\frac{100\ \text{mg/L} \times 10\ \text{mL}}{(10\text{mL} + 100\text{mL})} = 9.09\ \text{mg/L}$

2. Actual concentration of the spike + sample =

$$\frac{(\text{meter reading spiked sample}) \times (\text{volume of spike + sample})}{\text{original sample volume}}$$

3. % Recovery of spike =

$$\frac{(\text{answer from B}) - (\text{meter reading sample w/o spike}) \times 100}{\text{Answer from A.}}$$

TROUBLE SHOOTING

A. Standards and samples should be at room temperature before analysis.

B. The probe's membrane should be free of air bubbles while immersed in samples. Holding the probe at a 45 degree angle should prevent bubbles.

C. Unusually long periods of time for meter reading stabilization and membrane holes or discoloration may indicate the internal filling solution and membrane needs to be changed.

D. Ammonia concentrations less than 1 ppm need longer stabilization times, from 5 to 10 minutes, to obtain a more accurate reading.

E. If a power outage occurs while the meter is in standby or in operation, the memory may be lost. The meter will need to be re-programmed and recalibrated before the analysis can be continued. If this happens, unplug the meter to reset the electronics before proceeding with programming.

F. Never touch the membrane with the hands. Oils from the hand can coat the membrane and interfere with normal operation.

G. Final Effluent sample should be checked for Chlorine and dechlorinated if necessary.

H. For problems other than the ones mentioned, refer to the electrode manual (Model 95-12 Ammonia Electrode Instruction Manual) or instrument instruction booklet (Orion Bench Top pH/ISE Meter Instruction Manual Model 920A).

VII. INTERFERENCES

High concentrations of dissolved ions can affect the measurement of ammonia using this method. Amines are a positive interference. This may be enhanced by acidification. Mercury and silver interfere by complexing with ammonia.

VIII. PRECISION AND BIAS

See Section 4500-NH_3.A.4, Standard Methods 18th Ed.

IX. REFERENCES

A. Standard Methods for the Examination of Water and Wastewater, I8th Editions, A Section 4500-NH_3.A.4,PHA-AWWA-WEF, Washington, DC. (1992).

B. Orion Laboratory Products Group Bench Top pH/ISE Meter Instruction Manual, Model 920A, (1990).

Revision July 2000

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Determination of pH

Electrometric Technique

Reference: Standard Methods, 18th edition, Procedure 4500-H*B (19th and 20th edition references are also acceptable)

Apparatus:

1. pH Meter
2. Combination electrode
3. Stirring apparatus (optional)

Reagents:

1. Buffers, pH values 4, 7, and 10. Purchased commercially.

Sample Collection:

1. Samples are collected in clean plastic bottles and brought immediately to the lab for pH measurements.

Calibration:

These calibration instructions apply only to a sample pH meter. You should consult the manufacturer's instructions for directions on how to calibrate your pH meter. In all cases, the meter should be calibrated with two buffers that bracket the pH measured, and then verified with a third buffer that falls between the two used for calibration.

NOTE: If only occasional measurements are made, calibrate before each measurement.

1. Switch the Function knob to Standby and set then Temperature Control to the temperature of the buffer. NOTE: The buffers and samples must all be at the same temperature.

   NOTE: Not all manufacturers suggest stirring samples, but it can improve electrode response time and sample homogeneity. If samples are stirred, standards should be stirred in the same fashion. Some magnetic stirrers generate enough heat to increase the temperature of the sample. To avoid this, place piece of cardboard between the stirrer and the beaker.

2. Immerse the electrode into the **pH 4 buffer** and switch the Function knob to the pH mode. With the Normal/Expand knob in the Normal position, the meter should be adjusted to read **4.0**, on the normal scale, using the Calibrate knob.

3. Switch the Function knob to Standby, remove the electrode from the buffer. Rinse with distilled water.

4. Immerse the electrode into second buffer solution (suggested pH 10 so that the buffer bracket the pH measured).

5. Switch the Function knob to the pH mode. With the Normal/Expand in the Normal position, adjust the meter to read **pH 10.0** on the normal scale, using the Temperature Control.

   NOTE: If the second buffer requires more than a 2°C adjustment of the Temperature Control, re-check the temperature of the buffer solutions, or replace the buffer with fresh solution and repeat the calibration.

6. Switch the Function switch to Standby, remove the electrode from the solution and rinse with distilled water. Store the electrode in 4 M KCL. *The electrode storing solution varies with the type of electrode used. To prevent faster deterioration of the electrode (and more costs to the lab), store the electrode in the storing solution recommended by the manufacturer*.
7. Using a third buffer that falls between the two used for calibration (e.g.;**pH 7.0**), verify the accuracy of the calibration. Immerse the electrode into the **verification buffer** solution (**pH 7**, a value that falls within the calibration range).

8. Switch the Function knob to the pH mode. Wait for the reading to stabilize (usually no more than two to

three minutes. Read and record the pH value to the nearest 0.1 unit.

9. If the measured pH of the verification buffer falls within ±0.1 pH units of the expected value (i.e., 6.90 to 7.10), then the calibration is acceptable, and you can move forward with sample analysis.

   Note: *If your pH meter does not allow calibration with pH buffers that are more than 3 pH units apart, you can calibrate with the 4 and 7 and then check the calibration using either the 4, 7, or some other buffer between the two. Alternatively, for samples with pH values between 7 and 10, you can calibrate with the 7 and 10 and then check the calibration using either the 7, 10, or some other buffer between the two.*

Procedure:

1. Standardized the electrode as described above.

2. Place the sample in a clean glass beaker. Use a sufficient volume to cover the sensing elements of the electrode.

3. Immerse the electrode in the sample. Wait for the reading to stabilize (usually no more than two to three minutes.) Samples and pH buffer standards are not stirred during measurement. The stirring action causes carbon dioxide to be absorbed into the sample that can affect pH readings in samples that lack adequate buffering capacity (i.e., low alkanlinities).

4. Read and record the pH value to the nearest 0.1 unit.

5. Rinse the electrode with distilled water. Store in 4 M KCl (*or as recommended by the manufacturer*).

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Determination of Chlorine Residual DPD Colorimetric Method

Reference: Standard Methods, Procedure 4500 C1 G

Method number refers to 18th edition of Standard Methods, (19th and 20th edition references are also acceptable):

Sample Collection:

1. Collect sample immediately prior to returning to the lab.
2. Sample must be a grab.
3. Sample should not be mixed or unnecessarily agitated after collection.

Apparatus:

1. Fixed Wavelength Colorimeter

Procedure

CHLORINE, TOTAL

1. Fill a 10-mL sample cell to the 10-mL line with sample. Cap.

NOTE: Samples must be analyzed immediately and cannot be preserved for later analysis.
NOTE: Be sure the instrument is in the low range mode. See page 30.

2. Add the contents of one DPD Total Chlorine Powder Pillow to the sample cell (the prepared sample). Cap and gently shake for 20 seconds.

NOTE: Gently shaking dissipates bubbles that may form in samples containing dissolved gases.

3. Wait 3 minutes. During this period, proceed with Steps 4-8.

NOTE: A pink color will form if chlorine is present.
NOTE: Accuracy is not affected by undissolved powder.

4. Fill a 10-mL sample cell to the 10-mL line with sample (the blank). Cap.

5. Remove the instrument cap.

NOTE: For best results, zero the instrument and read the sample under the same lighting conditions.

6. Place the blank in the cell holder, with the diamond mark facing you. Tightly cover the cell with the instrument cap (flat side should face the back of the instrument).

NOTE: Wipe liquid off sample cells.

7. Press: **ZERO**

The instrument will turn on and the display will show - - - followed by 0.00.

*NOTE: The instrument automatically shuts off after 1 minute and stores the last zero in memory. Press **READ** in complete the analysis.*

8. Remove the cell from the cell holder.

9. Within 3 minutes after the 3-minute period, place the prepared sample in the cell holder.

NOTE: Wipe liquid off sample cells.

10. Cover the cell with instrument cap.

*11. Press: **READ***

The instrument will show - - - followed by the result in mg/L total chlorine.

NOTE: If the sample temporarily turns yellow after reagent addition, or shows over range (flashing 2.20) dilute a fresh sample and repeat the test. Some loss of chlorine may occur. Multiply the result by the dilution factor.

12. Record result for use in DMR.

Determination of Chlorine Residual Electrode (Iodometric) Method

Reference: Standard Methods, Procedure 4500 C1 I

Apparatus:

1. **Electrodes:** Either a combination electrode consisting of a platinum electrode and an iodide **ion-selective electrode, or two individual electrodes.**
2. **pH/millivolt ion meter:** Use an expanded scale pH/millivolt meter with 0.1 mV readability or a direct-reading selective ion meter.

Reagents:

1. **pH 4 buffer solution:** Dissolve 146 g anhydrous sodium acetate $NaC_2H_3O_2$ *or 243 g* $NaC_2H_3O_2 \bullet 3H_2O$ in 400 mL distilled water. Add 480 g concentrated acetic acid, dilute to 1 L with chlorine-demand-free water.

2. **Chlorine-demand-free water:** add sufficient chlorine to distilled or de-ionized water to give 5 mg/L free chlorine. After standing 2 days, this solution should contain at least 2 mg/L free chlorine; if not, discard and obtain better quality water. Remove remaining free chlorine by placing container in direct sunlight or irradiating with an ultraviolet (UV) lamp. Keep checking residual chlorine, do not use until all traces of chlorine have been removed. *Standard Methods provides discussion on how to store chlorine-demand-free water for extended periods*.

3. **Potassium Iodide (KI) solution.** Dissolve 42 g potassium iodide, KI, and 0.2 g sodium carbonate, $NaCO_3$, in 500 mL chlorine-demand-free, distilled water. Store in a dark bottle.

4. **Standard Potassium iodate solution (0.002 81 N):** Dissolve 0.1002 g potassium iodate (KIO_3)in chlorine-demand-free, distilled water, and dilute to 1000 mL. Each 1.0 mL, when diluted to 100 mL, produces a solution equivalent to 1.0 mg/L as Cl_2.

Calibration:

1. Prepare a series of iodate standards using the following table. Calibration should be performed any time reagents are changed.

Volume (mLs) Standard KIO_3	Final Volume (mLs)	Chlorine Equivalent (mg/L)
0	100	0.0
0.1	100	0.1
0.5	100	0.5
1.0	100	1.0

2. Pipet 1 mL acetate buffer and 1 mL KI solution into each flask (250 mL). Stopper, mix and let stand 2 minutes before diluting to volume (100 mL) with chlorine-demand-free, distilled water.

3. In order of increasing concentration – starting with the lowest concentration standard – pour the flask into a 150 mL beaker. Stir gently, without creating turbulence, and immerse electrodes.

4. Wait for the potential to stabile and record the potential in millivolts (mV).

5. Repeat this procedure for the other standards and then the reagent blank.

6. Prepare a calibration plot or regression as is done for ammonia (*refer to the Ammonia procedure*).

Sample Procedure:

1. Select a volume of sample not to exceed 100 mL that will not exceed the calibration range, and pipet into a 100 mL volumetric flask.

2. Pipet 1 mL of acetate buffer and 1 mL of KI solution into the flask. Stopper, mix and let stand 2 minutes.

3. Adjust sample pH between 4 and 5 if necessary, using acetic acid. Dilute to the mark with sample (if using 100 mL of sample) or with chlorine-demand-free water if using an aliquot of sample less than 100 mL. Stopper, mix, and let stand 2 minutes.

4. Pour the flask into a 150 mL beaker. Stir gently, without creating turbulence, and immerse electrodes.

5. Wait for the potential to stabilize and record the potential in millivolts (mV). If the millivolt reading exceeds that of the highest concentration calibration standard, repeat analysis with an appropiately smaller aliquot of sample.

Calculations:

1. Determine chlorine concentration in mg/L (A) from either the calibration plot, or a regression equation. Total residual chlorine is then calculated as:

$$\text{Total residual chlorine} = \frac{A}{V} \times 100$$

Where V = sample volume used (in mL). If total residual chlorine is below 0.2 mg/L, subtract apparent chlorine in reagent blank to obtain the true total chlorine residual.

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Determination of Total Phosphorus

Persulfate digestion followed by Ascorbic acid Method

Reference: Standard Methods, 18th edition, Procedure 4500-PB (5) & 4500 –PE

Apparatus:

1. Hot plate (An autoclave may be used in place of a hot plate.)
2. Bausch & Lomb Spectronic 100 spectrophotometer or equivalent
3. Cuvettes. If multiple cuvettes are used, they should be a matched set.

Note: Use only glassware designated for phosphorous analyses. Wash glassware with a phosphate-free detergent and 1:1 hydrochloric acid after each use.

Reagents:

1. **Phenolphthalein** indicator. Make phenolphthalein indicator solution by dissolving 0.1 g phenolphthalein in 50 mL of ethyl alcohol. Add 50 mL distilled water. Or purchase commercially prepared product.

2. **Sulfuric acid solution (30%):** Slowly add 300 mL conc. H_2SO_4 to approximately 600 mL distilled water that has been placed in a beaker or flask immersed in cold water. DO NOT ATTEMPT TO MIX THIS SOLUTION IN THE SULFURIC ACID BOTTLE. Allow to thoroughly cool and dilute to 1 L.

3. Ammonium persulfate: $(NH_4)_2S_2O_8$, crystalline

4. Sodium hydroxide: (NaOH), 1N.

5. **Stock Phosphate solution, 50 mg/L:** Dissolve 219.5 mg (02195g) potassium dihydrogen phosphate ($KH_2\ PO_4$) in reagent water. Dilute to 1 L. 1 mL = 50.0 ug (0.050 mg) PO_4^{-3} -P [as phosphorous].

   NOTE: It is recommended that KH_2PO_4 be purchased from two different suppliers. The material from one source should be used to prepare calibration stock standards, while the other source is used to prepare stock standards used to prepare spike samples. If the same solution that is used to prepare calibration standards is also used to prepared spiked samples, errors made in the preparation of the stock standard cannot be easily identified.(Commercially prepared standard solutions may be purchased with certified concentrations).

6. **Standard Phosphate solution, 2.5 mg/L:** Pipette 25.0 mL of the stock solution into a 500 mL volumetric flask.

7. **Potassium antimonyl tartrate reagent:** Dissolve 1.3715 g $K(SbO)C_4H_4O\bullet{}^1/_2\ H_2O$ in 400 mL reagent water in a 500 mL volumetric flask. Dilute to volume. Store in a glass-stopped bottle.

8. **Ammonium molybdate reagent:** Dissolve 20 g $(NH_4)_6Mo_7O_{24}$ in 500 mL reagent water in a glass-stoppered bottle.

9. **Ascorbic acid, 0.01M:** Dissolve 1.76 g ascorbic acid in 100 mL water. Store at 4°C. Discard after one week.

10. **Sulfuric acid, 5N:** Slowly add 70 mL conc. H_2SO_4 to approximately 500 mL distilled water that has been placed in a beaker or flask immersed in cold water. DO NOT ATTEMPT TO MIX THIS SOLUTION IN THE SULFURIC ACID BOTTLE. Allow to thoroughly cool.

11. **Combined reagent (Color Reagent):** For 100 mL combined reagent mix in this order 50 mL 5N sulfuric acid, 5 mL potassium antimonyl tartrate reagent, 15 mL ammonium molybdate reagent and 30 mL ascorbic acid. Let all reagents reach room temperature before combining. Mix the solutions well after each reagent is added. If the solution turns cloudy after mixing, let stand until clear. **This reagent must be used within four hours of preparation.**

12. **Color Blank Reagent:** *Prepare only if necessary*—see Sections 4 and 5. For 100 mL combined reagent mix in this order: 35 mL reagent water; 50 mL 5N sulfuric acid; and 15 mL ammonium molybdate reagent. Let all the reagents reach room temperature before combining. Mix the solutions well after each reagent is added. If the solution turns cloudy after mixing, let stand until clear. **This reagent must be used within four hours of preparation.**

Calibration:

1. Preparation of Standard Curve *Make a new standard curve every three months or when reagents are replaced or whenever a check standard is not within 10% of true value.* If a full set of calibration standards is not prepared on each day samples are digested, then at least one known standard prepared from the stocks used to prepare the calibration standards, must be made. If the result obtained for this known standard is not within 10% of the "true", or prepared, concentration, then a full calibration is required and samples must be re-digested.

   a. Prepare at least three standards plus a blank at concentrations that bracket the concentration of the sample measured. This analysis has been demonstrated to be substantially non-linear beyond 1.0 mg/L. Consequently, although some

newer spectrophotometers are able to extend the linear range, you should limit your calibration to an upper end of 1.0 mg/L.

b. Digest and test calibration standards in the same manner as the samples. Since the EPA is no longer requiring that calibration standards be digested, undigested standards are allowed provided that a mid-point knows standard is prepared and digested with each set of samples processed. If the recovery of this digested standard is not within 90% to 110%, there is indication that the digestion process significantly impacts results, and calibration standards should be digested as well. Plot absorbance vs mg/L phosphate in standard to give a straight line.

Note: The procedure in Standard Methods suggests that this line should be drawn through the origin.

2. Digestion:

- Hotplate Digestion
  Boil all treated samples, standards, and blanks for 30-40 minutes or until a final volume of 10 mL is reached.

- Autoclave Digestion
  Autoclave for 30 minutes in an autoclave or pressure cooker. Set the conditions for 15-20 psi. (98-137kPa) Samples are not to boiled dry. Note: *there is little or no volume reduction with this technique.* Keep this in mind when preparing matrix spikes.

With both techniques, samples, standards and blanks are allowed to cool following digestion. A drop of phenolphthalein indicator is added and the sample neutralized by adding 1N NaOH dropwise until a faint pink color is achieved (this will be pH 7.0$\pm$ 0.2). Dilute to 100 mL, but don't filter.

a. Pipet a suitable portion of thoroughly mixed sample into a 250 mL Erlenmeyer flask.

Note: As an example - use 2.0 mL of raw effluent , 10.0 mL of final effluent

b. Dilute to 50 mL (if less than 50 mL is used).

c. Pipette 50 mL of a standard into a 250 mL Erlenmeyer. It is best to vary the concentration of the standard as a check on different points on the calibration curve.

d. Prepare a blank using 50 mL reagent water.
e. Add one drop phenolphthalein solution to each flask. If a red color develops, use a dropper to add sulfuric acid solution (30%), one drop at a time, until the red color is gone.

f. Add one more mL of sulfuric acid solution (30%) to the flask.

g. Add one glass scoop (calibration to = 0.4g) ammonium persulfate. You may wish to pre-weigh out several aliquots of 0.4 g on disposable "weigh boats".

h. Put flasks on a hot plate. *Be careful when transporting beakers! To avoid contaminating samples with phosphorus, you should be wearing laboratory gloves. Do not contact the inside of the vessel.* Boil slowly for 30 to 40 minutes or until the volume in the beaker or flask is reduced to approximately 10 mL. (**Do not allow to go to dryness**. If samples boil dry, you must discard that sample and start over with fresh glassware.)

i. Remove the flask from the hot plate. Cool to room temperature.

j. Add distilled water to flask until the volume is approximately 30 mL.

k. Add one drop phenolphthalein solution.

l. Add sodium hydroxide solution with a dropper one drop at a time until a faint (light) pink color appears. Do not add excess NaOH.

m. Transfer the sample into a 100 mL volumetric flask; dilute to volume with reagent water.

3. Color Development

a. Pipet 50 mL digested sample into an Erlenmeyer flask. If you know or suspect the sample to contain appreciable concentration of phosphorus, use an aliquot of sample that has been diluted to 50mL with reagent water (e.g., if your expected sample concentration is 3 mg/L, you may wish to dilute 10 mL of digested sample to 50 mL with reagent water). Be sure to account for any such dilution when calculating sample results. Note: By "coloring", at most, 50 mL of the digested sample, you will have sufficient volume remaining to prepare a dilution if the sample response exceeds your calibration range.

b. Add 8.0 mL combined reagent to the 50 mL sample. Mix thoroughly.

c. Allow the color to develop for **at least 10 minutes but no longer than 30 minutes.**

d. Set the absorbance to zero using a digested reagent water blank. If any appreciable blue color is observed in this calibration blank, it should be noted, and corrective action should be initiated to identify the source of contamination.

NOTE: This procedure assumes that calibration standards are digested. If they are not digested, zero the spectrophotometer each day of analysis with an undigested (like the standards) reagent water blank to which color reagent has been added.

e. Wipe the outside of each cuvette with a Kimwipe or soft tissue before inserting into the spectrophotometer. Use the same cuvette for all blanks, standards, and samples. Different cuvettes may somehow be differentially dirty or scratched leading to differences in baseline. This leads to bias in the analytical data, or may affect the ability to meet quality control limits. If multiply cuvettes are used, they should be a matched set.

f. Rinse the cuvette between samples using the next sample to be tested.

g. The spectrophotometer should be set at 880 nm.

h. Read and RECORD the absorbance.

4. Calculate phosphorus concentration in sample as follows:

$$\text{mg/L total P} = \text{mg } \frac{\text{P (from curve)}}{\cancel{\text{L}}} \times \frac{\text{V}\cancel{\text{mL}}}{1000\cancel{\text{mL}}} \times \frac{1\text{L}}{} \times \frac{\text{FV}\cancel{\text{mL}}}{\text{CV}\cancel{\text{mL}}} \times \frac{1}{\text{SV}\cancel{\text{mL}}} \times \frac{1000\cancel{\text{mL}}}{\text{L}}$$

Where
V =volume (mL) of sample + reagent water that was colored [typically 50]
FV =final volume (mL) after digestion [typically 100]
CV =volume (mL) of sample that was colored [typically50]
If you used 10 mL diluted to 50 with reagent water, A=10
SV =original volume of sample that was digested [typically 50]

By canceling out units, this formula can be simplified to:

$$\text{mg/L total P} = \frac{\text{mg P (from curve)}}{\text{L}} \times \frac{\text{VxFV}}{\text{CVxSV}}$$

NOTE*: If you digest 50 mL of sample, dilute to a final volume of 100 mL. take 50 mLs of the digested sample and color it, the equation simplifies to*:

$$\text{mg/L total P} = \frac{\text{mg}}{\text{L}} \text{P (from curve)} \times 2$$

5. The use of "color" blanks (if the sample has appreciable color following digestion)
Some plants analyze samples that seasonally develop color due to algae or other things. This type of color in a sample will register background absorbance on the phosphorus analysis and therefore must be subtracted from the true sample signal. This requires determining the absorbance of a "color blank".

A second aliquot of the digested sample (without the addition of combined reagent solution) should be read, recording the absorbance. This value should be subtracted from the absorbance obtained from the aliquot of the same digested sample to which combined reagent has been added.

Follow the procedure in steps 3 a and 3 b. It is important to use precisely the same amount of digested sample for "color blank" as used for the actual sample analysis.

Sample Calibration and Limit of Detection (LOD) Data from a typical WWTP:
Calibration Data

Concentration	Absorbance
0	0
0.25	0.058
0.5	0.130
1.0	0.291

Slope= 3.38212347
Intercept= 0.03249071
Correlation coefficient= 0.99765256

$$\text{Concentration} = \frac{\text{Absorabance} - \text{Intercept}}{\text{Slope}}$$

LOD Determination

Total Phosphorus

Spike level= 0.1 mg/L
Rep.1 0.11
Rep.2 0.12
Rep.3 0.12
Rep.4 0.12
Rep.5 0.12
Rep.6 0.11
Rep.7 0.12

# replicates	t-value
7	3.143
8	2.998
9	2.896
10	2.821

mean 0.11714
st. dev 0.00488
t-value 3.143= from table based on # replicates
MDL 0.01534= t-value x std deviation
LOD 0.01534= roughly= to MDL
LOQ 0.05112= 10/3x the LOD

The 5-point check

1 Is the MDL no lower than 10% of the spike level? yes
2 Is the spike level greater than the calculated MDL? yes
3 Is the MDL below any relevant permit limit? N/A
(if there is one) Permit limit?
4 Is the signal-to-noise ratio (S/N) between 2.5 and 10? CHECK
S/N = Mean/std dev.
S/N is fairly high that suggests a need to spike at a lower concentration
5 Is mean recovery within reasonably expected limits? yes
Mean recovery= mean/spike level x 100 117.14%

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COLISCAN®MF Membrane Filter Procedure

For the differentiation and enumeration of *Escherichia coli*

Indiana Department of Environmental Management

August 2001

I. Introduction

This alternate membrane filter technique utilizes chromagenic substrates for galactosidase and glucuronidase to identify bacteria. Organisms, that produce galactosidase but not glucuronidase, grow as pink-magenta colonies, while organisms, that produce glucuronidase but not galactosidase, grow as green-teal blue colonies. **Most strains of *E. coli* produce both enzymes, resulting in blue to purple colonies** visible as early as 18 hours (35°C incubation). *E. coli* is visible earlier than other bacteria due to its fast generation time and utilization of both chromagens instead of only one or the other as is the case with most other bacteria. This technique allows the recovery of weakened organisms and readable results at 22 +/- 2 hours.

II. Sampling, Storage and Preservation

Samples should be collected in the same manner as those for fecal coliform determination in surface and bathing waters. If samples can not be analyzed within 1 hour after collection, they must be held on ice during transport to the laboratory. Hold temperature of all samples below 4°C during a maximum transport time of 6 hours. Refrigerate these samples upon receipt in the laboratory and process within 2 hours.

III. Apparatus

A. Reagents

1. Buffered Dilution Water

Composition:

Sodium Dihydrogen Phosphate ($NaH_2PO_4 \bullet H_2O$)	0.58 g
aka(Sodium phosphate, monobasic, monohydrate)	
Sodium Monohydrogen Phosphate (Na_2HPO_4)	2.50 g
aka(Sodium phosphate, dibasic, anhydrous)	
Sodium Chloride (NaCl)	8.50 g

Preparation:

Dissolve the ingredients in 1 L. of distilled or deionized water preparing sufficient volumes for use as rinse water. Autoclave after preparation at 121°C (15 lbs. pressure) for 15 minutes. Final pH should be 7.4 +/- 0.2.

2. Coliscan® Broth (1.75-2.0 mL per plate)

3. 95% Methanol for flaming

B. Equipment

1. Filter apparatus
2. Membrane Filters, 48 mm, 0.45 mm pore size
3. Plastic petri dishes with pads, 9 X 50 mm with tight lids
4. Sample bottles
5. Incubator (35° +/- 0.5°C)
6. Forceps
7. Bunsen Burner
8. Dissecting Scope (at least 10 to 15 X)
9. Autoclave

IV. Procedure

A. Filtration

1. Prepare appropriate dilutions, remembering that *E.coli* populations may be equal to or higher than other fecal coliform populations.

2. Set-up sterile apparatus.

3. Dispense 1.75 – 2.0 mL. Coliscan® broth into each petri dish and label accordingly, including duplicates, Q_A, Q_B, Pos Q_C.

4. Turn on aspirator.

5. Light Bunsen burner.

6. Dip forceps in methanol and flame to sterilize.

7. Tear open the pre-sterile filter.

8. With the sterile forceps carefully pick out the filter, lift off the funnel, place filter in the center of the base and replace funnel.

9. Shake the sample bottle vigorously to obtain an even distribution of organisms in the sample.

10. If less than 20 mL. of sample is to be filtered, clamp off the filtration unit, and add approximately 20 mL of buffered water then pipette appropriate sample volume into the funnel. Remove the clamp and allow to filter.

11. Rinse with no less than 25 mL. of buffered water three times. Be certain to rinse down the sides of the funnel to prevent organisms from clinging to the sides of the apparatus.

12. Remove funnel

13. Dip forceps into methanol and flame to sterilize.

14. Grasp filter membrane containing organisms with sterile forceps and place on absorbent pad containing Coliscan® broth by first allowing one edge to make contact and then carefully rolling it onto the surface to avoid entrapment of air between the filter and the nutrient medium.

15. Close plate and secure lid.

16. Repeat steps 6-16 for all samples, duplicates and dilutions.

B. Incubation

1. Place plates **upside down** into a 35°C incubator.

2. Incubate for 22 hours +/- 2 hours.

C. Enumeration and identification

1. Remove plates from incubator.

2. Open and count all blue and purple colonies (except pinpoint colonies less than 0.5 mm. diameter should not be counted).

V. Quality Control

A. Duplicates of each dilution should be analyzed.

B. Q_A and Q_B plates should be run always as the first and last sample in a series. They are simply 100 mL. of buffered water analyzed as a sample. These are indicators of sterility and rinsing techniques.

C. A positive Q_C plate, that is always the next to the last sample and helps to identify adequate growth conditions as well as rinsing technique. Prepare a dilution of *E.coli* from a pure culture (or use one drop of primary effluent) to yield a countable plate.

Filter and incubate as you would a sample.

D. A countable plate is one with *E.coli* colony numbers between 20 and 80. Any plates above this range should be designated as TNTC (too numerous to count). To insure against the possibility that all dilutions will be TNTC, a 1 mL. dilution should be run with each test in addition to the dilutions that regularly produce countable plates. (In the unlikely event that even the 1 mL. dilution is judged TNTC, see IDEM letter dated January 3, 2001 regarding TNTC reporting.)

VI. Calculations

Multiply the number of *E.*coli colonies on the plate by the dilution factor. This calculated number represents the number of E. coli/ 100 mL. of sample.

VII. Precision and Accuracy

VIII. Troubleshooting

Count only the blue and purple colonies. Some operators may initially be confused in differentiating between purple and pink colonies as purple/blue colonies are a combination of the product of the color from both enzyme substrates (green-teal and pink-magenta). Normally, the purple/blue colonies appear first on the membrane because they contain color from both substrates, and the pink are very much lighter colored. However for operators who are unsure of the colors, Micrology Lab has available test media to differentiate whether the color is from only a single substrate (not *E.coli*) or a combination of both substrates (*E.coli*). This is normally included with full instructions with the first orders of Coliscan® MF, but can be ordered as a separate item from Micrology Laboratories (Cat. #25101 Confirmation Media).

IX. Interferences

X. References

XI. Updates

Detection and Enumeration of *E.Coli* in wastewater utilizing Colilert® and Quanti-Tray®/2000

9223 B. Enzyme Substrate Test

BACKGROUND INFORMATION:

Colilert® was originally developed for use in clinical laboratories to enumerate and identify bacteria in urine. It was then adapted for the simultaneous detection and confirmation of total coliforms and *E.coli* in the analysis of drinking water.

PRINCIPAL:

As total coliforms and *E.coli* bacteria metabolize the nutrient indicators in Colilert, that produces two distinct reactions. The two nutrient indicators are: (1) o-nitrophenyl- $-D-galacto-pyranoside that changes from clear to yellow for total coliform and (2) 4-methylum-belliferyl-$-D-glucuronide that will emit a definite blue fluorescence when using a long-wavelength 6 watt, 366 *nm* ultraviolet light for *E.coli*.

APPARATUS:

A. Reagents:

WHEN PERFORMING ALL TESTS USE APPROPRIATE TECHNIQUES AND TAKE ALL SAFETY PRECAUTIONS NECESSARY. REFER TO THE MSDS ON FILE FOR ADDITIONAL SAFETY INFORMATION.

1. Colilert© Reagent-Snap Pack [IDEXX Laboratories]- for 100 mL water samples. Shelf life: up to 12 months from date of manufacture (see box or individual packets for specific expiration date). Store at 4-30°C.
2. Color Comparator [IDEXX Laboratories] is a liquid color reference reagent used to distinguish a minimum positive from negative test results at 24 hours (for Quanti-Tray 2000).

B. Equipment:

- Non-fluorescing sample bottles-sterilized containing sodium thiosulfate or equivalent
- 6 watt long wave (366-nm) UV Lamp
- UV Absorbing safety glasses
- Incubator (35 ± 0.5°C)
- Autoclave
- Quanti-Tray Sealer [IDEXX Laboratories]
- Quanti-Tray/2000, (97-wells) [IDEXX Laboratories]

PROCEDURE:

When performing all tests use aseptic techniques. Take all safety precautions necessary.

Procedural Notes:

- Avoid touching the top of the media pack after it has been opened or the inside of the sample bottle or cap.
- Avoid prolonged exposure of the inoculated Colilert to direct sunlight. The indicator compounds may be hydrolyzed, creating a false positive total coliform result.
- Upon adding Colilert to the sample, a transient blue color may appear in samples containing an excessive amount of free chlorine.

A. QC FOR PURCHASED PRODUCTS:

Performing Quality Control on each lot of media, bottles and trays will eliminate QC on each sample run. When ordering for the year, ask for all of one lot number so that numerous QC analyses will not have to be done.

1. Warm up the sealer per manufacturer's instructions.
2. To each of three sterile bottles, add 100 mLs. of sterile water and label them with the following culture IDs. Inoculate each one with the corresponding cultures:

Culture	Expected Result
E.coli (ATCC 25922, or 11775)	yellow, fluorescent
Klebsiella pneumoniae (ATCC 31488)	yellow, not fluorescent
Pseudomonas (ATCC 10145 or 27853)	clear, not fluorescent

3. Add one pack of reagent to each of the samples. "Flip" the pack to loosen the media, then snap open the top and pour aseptically into the sample bottle.
4. Cap and shake the sample. Wait a few minutes for the reagent to dissolve.
5. Label each Quanti-Tray/2000 with the date time and culture ID.
6. Re-shake the sample, remove the cap and take the corresponding Quanti-Tray/2000, carefully squeezing on the outer edges of the tray to open it and pour the sample into the Quanti-Tray. Run the sample through the tray sealer, the sealer will dispense the sample into the wells and seal the package.
7. Run the sample through the tray sealer, the sealer will dispense the sample into the wells and seal the package.
8. Incubate for 24 hours at (35 ± 0.5°C].
9. Read the Quanti-Tray for color and fluorescence as indicated in step 2 above.

NOTE: If a tray does not match the expected results, check for errors in technique. Such as: contamination of the bottle or Quanti-Tray during procedure, transferring the culture to an incorrectly labeled bottle. Repeat procedure. If it fails again call the manufacturer to see if there has been problems with those particular lots. Record all lot numbers and results.

The State Department of Health only requires one check of the Quanti-trays per lot for drinking water. As a further check on sterility of the Quanti-trays, a blank, using sterile water, may be analyzed monthly.

B . SAMPLE COLLECTION

Collect the *E.coli* sample at the same time and place for the final effluent Total Residual Chlorine sample**.** Collect a 100 mL. sample in a sterile bottle containing sodium thiosulfate. Take the sample directly to the lab and analyze immediately or sample can be refrigerated for up to 3 hours before analysis.

C. SAMPLE ANALYSES: MULTI-WELL PROCEDURE

1. Warm up the sealer per manufacturer's instructions.
2. In a sterile sample bottle containing 100 mL of sample add one pack of reagent. "Flip" the pack to loosen the media, then snap open the top and pour aseptically into the sample bottle.
3. Cap and shake the sample. Wait a few minutes for the media to dissolve.
4. Label each Quanti-Tray with the date time and culture ID.
5. Re-shake the sample, remove the cap and take the corresponding Quanti-Tray, carefully squeezing on the outer edges of the tray to open it and pour the sample into the Quanti-Tray.
6. Run the sample through the tray sealer, the sealer will dispense the sample into the wells and seal the package.
7. Record all pertinent information, analyst initials, date, time and sample ID on the bench sheet.

D. INCUBATION

Place in a 35± 0.5°C incubator for 24 hours Record daily in a logbook.

E. ENUMERATION AND IDENTIFICATION

1. After 24 hours of incubation, remove the trays from the incubator. Use the color comparator to compare the yellow and fluorescent signal. Sample wells that are clear (no color change) are negative for total coliform and *E.coli*. All yellow wells, greater than or equal to the comparators are counted as positive for total coliform. Next examine all total coliform-positive wells for fluorescence using the 366 mn UV lamp. Yellow and blue-fluorescent wells, greater than or equal to the comparator, are counted as positive for *E.coli*. Record the number of large and small wells for Quanti-Tray/2000.
2. Samples incubated over 28 hours use the following guideline: if no yellow color is present the sample is negative. A yellow color or fluorescing is a VOIDED sample.

F. *CALCULATIONS:*

1. Use the MPN (Most Possible Number) table provided by IDEXX and determine the MPN/100 mL for *E.coli.*
2. For Quanti-Tray/2000, find the number of small wells across the top of the Table. Then go down the left side of the chart and find the number of large wells. The MPN/100 mL value is where the value for the large and small wells intercepts. MPN is a number, based on certain probability formulas and is an estimate of the mean density of coliforms in the sample.
3**. If any dilution of the sample is made, multiply the MPN/100 mL value by the dilution factor to obtain the final MPN/100 mL value.**
4. Record results on the appropriate bench sheet. Initial, date and time for all results.

REFERENCES:

Standard Methods 18th ed. 9221D. Pp9-51 through 9-52.
Standard Methods 20th ed. 9223 Pp9-68 through 9-70.
IDEXX Laboratories, Inc. One IDEXX Drive, Westbrook, ME 04092

SAMPLE BENCHSHEETS

Biochemical Oxygen Demand

Five Day Carbonaceous

	IN	OUT
Analyst:	_____	_____
Temperature:	_____	_____
Barometric Pressure:	_____	_____
Time:	_____	_____

Date of Sample Collection:_____
Day & Date of Analysis:_____
Day & Date Out:_____

SAMPLE ID	BOTTLE ID	mL SAMPLE	mL SEED	INITIAL DO	FINAL DO	DEPLETION	LESS SEED	DILUTION	cBOD (mg/L)	AVERAGE
Blank 1										
Blank 2										
Seed 1										
Seed 2										
STD										
Final 1										
Final 2										
Final Dup										
Pri Eff 1										
Pri Eff 2										
Raw 1										
Raw 2										
Raw Dup										

Dechlorination_____Drops of Na_2SO_3 per 100 mL Cl_2 Residual after Dechlorination_____Positive_____Negative

Analyses for cBOD are performed according to Standard Methods, 18th Ed., Method 5210B

TOTAL SUSPENDED SOLIDS

Date of Collection_____
Balance Calibration:
Low Wt:_____g
Medium Wt:_____g
High Wt:_____g

Date / Time of Analysis:_____

Analyst:_____ On Computer?:_____
QC Recorder?:_____

SAMPLE NAME	RAW	RAW DUP	THKOV	PRIEFF	ML #1	ML #2	ML #3	ML #4	ML #5	RAS
Crucible No										
Volume (mL)	**25**	**25**		**50**	**2**	**2**	**2**	**2**	**2**	**2**
Dry Wt.										
Crucible Wt.										
Ash Wt.										
TSS (mg/L)										
VSS (mg/L)										

Sample Name	FINAL	FINAL DUP	GCI	M196	BLANK	CONSTANT				
Crucible No										
Volume (mL)					**250**					
Dry Wt.										
Crucible Wt.										
Ash Wt.										
TSS (mg/L)										
VSS (mg/L)										

-Analysis for TSS and VSS are performed according to Standard Methods, 18th Ed., Methods 2540D & 2540E respectively.

AMMONIA NITROGEN – Electrode Method

DATE / TIME:_____
ANALYST:_____

SAMPLE IDENTIFICATION	SAMPLE DATE	METER READING (MV)	SAMPLE VOLUME	DILUTION FACTOR	CONCENTRATION mg/L
1 PT. CALIBRATION					
2 PT. CALIBRATION					
3 PT. CALIBRATION					
SLOPE					
BLANK					
3 PPM STANDARD					

Spectrophotometric Analysis

Analysis **Phosphorus** **Analysis Date** _____

Wavelength **880 nm** **Analyst** _____

Date	Sample Type	Abs ☐ Trans ☐	Initial Concentration	Dilution Factor	Final Concentration (mg/L)	% Rec
	Blank			1		
	0.2			1		
	0.4			1		
	0.6			1		
	Raw			25		
	Raw – Dup			25		
	Prim Inf			10		
	Prim Eff			10		
	Final			2.5		
	Final – Dup			2.5		
	Raw			25		
	Raw – Spike					
	Prim Inf			10		
	Prim Eff			10		
	Final			2.5		
	Final – Spike					

Spectrophotometric/Colorimetric Analysis

Analysis: Total Residual Chlorine _____ **Analysis Date** _____

Wavelength _____ **Analyst** _____

Date	Sample Type	Abs ☐ Trans ☐	Initial Concentration	Dilution Factor	Final Concentration (mg/L)	% Rec

E. Coli

Analysis Date:_____
Analyst In/Out:_____/_____
Incubation Time In: _____
Incubation Temperature 35 +/- .2

Location:_____
Procedure: _____
Time Of Collection: _____
Chlorine Residual:_____
Flow:_____

Sample Volume (mL)	Dilution Factor	Dish Label	# Colony Growths	# Colonies / 100 mL	Avg. # Colonies / 100 mL
			1.		
			2.		
			1.		
			2.		
			1.		
			2.		
			1.		
			2.		
			1.		
			2.		
			1.		
			2.		
100	1	Qa			
100	E. Coli (-7)	+			
100	1	Qb			

Colonies / 100 mL _____

pH

Analyst (s):_____

Date of Analysis _____

SAMPLES	RAW	RAW DUP	ML # 1	ML # 2	ML # 3	ML # 4	ML # 5	RAS	FINAL GRAB
DATE									
TEMP(^{o}C)									
TIME									
pH (S.U.)									

SAMPLE ID/ NAME									
DATE									
TEMP(^{o}C)									
TIME									
pH (S.U.)									

pH Calibration: 1 Std: _____ 2 Std: _____ 3 Std: _____ Slope: _____

pH 7 Std check: _____ pH 7 Std a.m. check _____ pH 7 Std p.m. check _____

-pH analyses are performed according to Standard Methods, 18 th Ed., Method 4500-H+B

Colilert (*E.coli)*

⇦ ***Collected*** ⇨⇦ ***Sample Information*** ⇨⇦ ***Set*** ⇨⇦ ***Results*** ⇨ ⇨ ⇨ ⇨

Date	**Time**	**Analyst**	**Chlorine Residual**	**Sample ID**	**Time**	**Analyst**	**Date**	**Time**	**Analyst**	***Large Yellow Fluorescing***	***Small Yellow***	**MPN**

Chain of Custody

Chain of custody (COC) in any laboratory is critical to ensure the validity of the samples collected. This is true even in the smallest of facilities. A record that includes the following information must be maintained for each sample collected:

- ❒ Sample location;
- ❒ Sample date;
- ❒ Sample time;
- ❒ Sampler (s) names or initials;
- ❒ Sample identification (either a unique I.D. number or the sample name, location, date and time)*;
- ❒ Preservation method (was there an addition of acid, sodium hydroxide, or other preservative etc., record volume added, pH verification, date and time of addition, refrigerated at 4ºC?)

*If any method but this is used, the sample information must be recorded and maintained in a separate log book. If this method is used (which is the case in most small operations when all work is performed in house) then a simple check list with a place for the date, analyst and a list of expected samples should be used for every day samples are received. (See Sample Bench Sheet section)

In any case, a written procedure must be used that describes how your particular laboratory tracks samples ensuring the proper preservation, container and holding times are met. Other required information includes a description how your COC procedure prevents samples from being mixed up with others, disposed before all analyses are completed or forgotten.

It is important to remember, regardless of the size of your facility, the number of people in your lab (even one) or the number of samples received that a ***written*** COC (Chain of Custody) procedure must be available. It is our responsibility as laboratory analysts to ensure the defensibility of sample handling and analysis. The COC is a critical step in that process.

BEST LAB IN INDIANA

Sample Receipt Checklist

Sample Date_____ Analyst_____ Today's Date_____

	Received Yes	No
Final Effluent (unpreserved)	❒	❒
(preserved)	❒	❒
Raw Influent (unpreserved)	❒	❒
(preserved)	❒	❒
Mixed Liquor	❒	❒
RAS	❒	❒
Digested Sludge	❒	❒
Primary Sludge	❒	❒
Final Effluent Grab (pH, Ecoli, Chlorine)	❒	❒
Other_____	❒	
Other_____	❒	
Other_____	❒	

Comments:

_____

_____

_____

CHECKLISTS

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BIOCHEMICAL OXYGEN DEMAND:
METHOD EVALUATION CHECKLIST
Method 5210-B, Standard Methods, 18th ed.

I. EQUIPMENT

A. Dissolved Oxygen Measurement

___1. The laboratory measures dissolved oxygen by:

__ Winkler titration

__ D.O. meter and probe

___2. Calibration and maintenance of D.O. Meter

__Calibration against air of known DO concentration.

__Calibration against water of known DO concentration (determined by iodometric method).

__Meter zeroed with saturated sodium sulfite solution.

__Electronic zero checked.

__Manufacture's procedure followed exactly.

__Membrane is replaced as necessary, and a record of maintenance kept.

__Air bubbles are excluded from under or around membrane when reading meter.

__The sample is adequately mixed during measurement.

___3. Winkler Titration

__Cross contamination of Winkler reagents is prevented; air bubbles are excluded.

__KI and Manganous Sulfate precipitate is allowed to settle.

__After addition of 1 mL conc. sulfuric acid, solution is mixed until precipitate is dissolved.

__The sample is titrated to a pale yellow straw color using a starch indicator.

B. Incubator

-- 1. The incubator temperature is kept at 20+1oC when in use.

-- 2. The lab keeps a record of incubator temperature.

-- 3. The lab keeps a record of incubator maintenance.

-- 4. Samples are incubated in the dark.

C. Glassware

-- 1. The laboratory uses bottles of 300 mL capacity, with ground glass stoppers.

-- 2. Large bore volumetric pipets are used for samples directly pipetted into BOD bottles.

-- 3. Glassware is available for making dilutions. (ie,1:1, 1:10, etc.).

-- 4. BOD bottles are cleaned with a detergent, rinsed thoroughly and drained before used.

-- 5. BOD bottles are rotated, i.e. used to hold different samples after each analysis.

II. REAGENTS

0.025 N Sodium Thiosulfate or 0.025 N Phenylarsine Oxide Titrant (Winkler Method) is:

-- 1. Purchased commerically or

-- 2. Prepared in the lab b y dissolving 6.205 g Na2S2O3•5H2O and 4.0 g NOH in 1L distilled.

-- 3. Standardized with potassium bi-iodate solution before use (if prepared in lab).

-- 4. Documented when standardized (if prepared in lab)

A. Standard potassium bi-iodate (Winkler Method)

-- 1. 0.021 M solution: dissolve 812.4 mg KH(IO3)2 in distilled water and dilute to 1L.

-- 2. Used for the standardization of sodium thiosulfate.

B. Starch Indicator Solution (Winkler Method)

-- 1. Prepared in the laboratory

-- 2. Purchased commercially

C. Neutralizing and dechlorinating reagents:

--1. Fresh Na_2SO_3 is prepared daily for neutralizing chlorine (chlorinated effluents only).

--2. 1 N H_2SO_4 for neutralizing high pH samples.

--3. 1 N NaOH for neutralizing low pH samples.

D. Nutrients for dilution water:

--1. Prepared in a laboratory: (list)_____
--2. Purchased commercially: (list)_____
--3. Discarded if there is any sign of biological growth in stock bottle.
--4. The pH of the phosphate buffer solution is 7.2.

E. Dilution water is:
--1. Made from: ___Distilled ___Deionized ___Other(specify)_____.
--2. Equilibration to 20^{o}C when is use.
--3. Protected by using clean glassware, tubing and bottles.
--4. Saturated with oxygen before use: __By shaking __By filtered aeration.
--5. Stored with a permeableplug.
--6. Prepared by adding 1 mL of each of nutrient solution per liter of water.

III. PROCEDURE

A. Pretreatment Steps
__1. Are samples checked for pH?
__2. If samples contain caustic alkalinity or acidity, are they:
__Neutralized?
__Seeded?
__ 3. If samples were disinfected, are they
__Checked for chlorine?
__Neutralized with Na2SO3
__Seeded?
__4. Are chlorinated and industrial effluents seeded even though no residual chlorine detected?

B. Transfer & Dilution Steps
__1. Are samples well mixed before pipetting?
__2. Are sampled warmed to 20 C berfore pipetting? (If were in cooler)
__3. Is samples prepared within 2 hours of collection if not cooled?
__4. Are at least two dilutions set up per sample?(3 is preferable)
__5. Are dilution water blanks set up for each analysis day and for each bottle of dilution water?
__6. Are sample volumes less than 3 mL diluted in a graduated cynlinder of volumetric flask before making final dilution in the BOD bottle?
__7. Are additional nutrients added to bottles when using a small volume of dilution water?

C. Seed
__1. Are seed controls set up when samples are seeded?
__2. Is the seed:
__added to the dilution water?
__added directly to BOD bottle?
__never used, except for GGAs, blind standards and reference samples?
__3. Is the seed made from:
__an unchlorinated, non-toxic effluent from the STP or
__a supernatant of settled raw wastewater?
__other (specify)_____.
__4. Do at least two seeded blanks deplete at least 2 mg/L with a residual of 1 mg/L after 5 days? If not,
__5. Does lab the lab calculate the seed correction factor with only those bottles meeting the depletion

D. Initial DO & Bottle Status
__1. Is an initial DO measured on every sample dilution, after set up and before incubation?
__2. If an initial DO> 9 mg DO/L at 20°C, is sample stripped of excess DO by agitation or aeration?
__3. Does analysis insure that no air bubbles are present in BOD bottle before incubation?
__4. Are water seals on bottles protected to prevent drying.
__5. Are BOD bottles labeled for sample ID?

E. Five Day Residual DO
__1. Is the residual DO after 5 days at least 1 mg/L?

__2. Is the depletion after 5 days at least 2 mg/L?
__3. Are BOD calculations does correctly?
__4. Are seed corrections used correctly?

QUALITY CONTROL/QUALITY ASSURANCE AND DATA

A. Known Standards Used:
__1. Laboratory prepared glucose-glutamic acid
__Are reagents dried for at least 1 hour at 103oC?
__2. Commercially prepared glucose-glutamic acid
__3. Other (specify)_____

B. Control Limits:
__1. Do the 5 day BODs for the GGAs fall within established control limits (198±30.5 mg/L)?
__2. Are limits established for replicates?
__3. Do the 5 days BODs for other standards fall within established control limits?
List these standards and their limits:_____

C. Data Reporting and Interpretation

__1. Is the DO uptake of dilution water blanks always 0.2 mg DO/L or less?
__2. Are corrective actions taken when DO uptake of dilution water blanks exceeds 0.2 mg DO/L?
__3. Do at least two sample dilution deplete at least 2 mg/L with a residual of 1 mg/L?
___If not, does the lab change the dilutions used to meet this criteria?
__4. Is the average BOD calculated using only those bottles meeting the depletion criteria?
__5. What BOD value is reported when duplicates are set:
__The highest
__The lowest
__The average
__Other (specify):_____
__

AMMONIA NITROGEN, ION SELECTIVE ELECTRODE:
EPA METHOD 305.3 EVALUATION CHECKLIST
(Method 4500-NH3 F&G, Standard Methods, 18th ed.)

Laboratory Name:_____
Date: _____

I. EQUIPMENT

___A. Meter and Probe
___ Specific Ion Meter with a direct concentration readout
___Ammonia Selective Electrode (Orion model 95-12 or equivalent)

___B. Insulated Magnetic Stirrer and Teflon Stir Bar

___C. Glassware
___Class A volumetric pipets and Class A volumetric flasks
___ 150 mL beakers, graduated cylinder

II. REAGENTS

___A. Ammonia-free water is:
___ Prepared by passing distilled water through an ion exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anions exchange resin.
___ Prepared by adding 1 mL concentrated sulfuric acid or chlorine to distilled water and distilling.
___Replaced when high blank values are obtained.
___ Protected from atmospheric contamination.
___Used to dilute all standards, reagents and samples.

___B. Sodium Hydroxide (NaOH), 10 N
___ Prepared in the lab by dissolving 400 g NaOH to 800 mL ammonia-free-water, cooling and diluting to 1 L.(Or same proportions of NaOH and water)
___Purchased commercially. (Orion Ammonia ISA, Cat # 951211 or equivalent)

___C. Stock Ammonium Solution Chloride Solution (NH_4CL), 1000 mg NH_3-N/L (1.00 mL= 1.00 mg N)
___Purchased commercially (Orion 951007 or equivalent)
___Prepared in laboratory: dried at 100°C before weighing 3.189 g anhydrous NH_4Cl per liter water.

___D. Standard Ammonium Solution (NH_4Cl), 10 mg NH_3-N/ L (1.00 mL= 0.01 mg N)
___ Purchased commercially
___Made from 10 mL Stock Ammonium Chloride (100 mg NH_3-N/ L) in 1 L water.
___Made from 10 mL of 100 mg NH_3-N/ standard solution in 100 mL water.

III. PROCEDURE

___A. Sample Preparation
___1. Confirm sample has no chlorine residual.

___ 2. Warm sample to room temperature before analysis.

___ 3. Working standards are prepared by diluting stock or standard solutions with volumetric glassware.

___4. If a Specific ION Meter is used:
___the meter is calibrated using at least two standards and a blank.
___The standards bracket the concentration range of interest.

___5. The temperature of standards and samples remain the same during calibration and testing.

___6. The same mixing rate is maintained for samples and standards remembering there should be no vortex when mixing.

___7. The manufacturer's instructions for the specific ion meter are followed exactly.

___8. A 3 ppm standard should be checked periodically throughout the analysis to verify the linearity of the calibration.

___9. Standards are read in order from lowest concentration to the concentration highest.

___ 10. A blank (not recorded) should be run between the standards and the samples to allow the sensor to approach zero.

___ 11. 100 mL of sample or ammonia standards is measured into a 150 mL beaker.

___12. 1 mL of 10 N NaOH or ISA is added to all standards and samples to pH>11 after electrode is immersed in the solution.(This prevents loss of ammonia gas).

___13. If more than 1 mL of 10 N NaOH is needed to raise the pH above 11, the volume is recorded and used in the calculations.

___14. Samples and standards are stirred slowly so that air bubbles are not sucked into solution and form on the membrane.

___15. The electrode is allowed to stabilize before recording concentration or millivolt values.

___16. The electrode is rinsed between each standard and sample with ammonia-free water.

___17. The electrode is stored according to manufacturer's guidelines.

___18. If distillation of samples is omitted, comparability data is on file for distilled vs. undistilled samples.

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TOTAL SUSPENDED SOLIDS, DRIED AT 103-105°C
EPA METHOD 160.2 EVALUATION CHECKLIST
(Method 2540- D, Standard Methods, 18th ed.)

EQUIPMENT

___A. Drying Oven, for operation at 103 to 105°C or,
___B. Analytical Balance, capable of weighing to 0.0001 gram
___C. Desiccator
    ___active colored desiccant indicates that the drying capacity is not exceeded.
    ___desiccator cover is sealed tightly.
___D. Glass-fiber filters used:
___E. Filter Holder, Gooch crucible adapter or membrane filter funnel.
___F. Gooch crucible, 25 to 40 mL capacity, suitable for filter size selected.
___G. Suction flask, of sufficient capacity for sample size used.
___H. Weighing dishes, if the membrane filter funnel is used.
___I. Tongs or forceps
___J. Vacuum source

PROCEDURE

___A. Preparation of Glass Fiber Filter
    ___1. Filters are seated with wrinkled side up.
    ___2. Filters are washed with 3 successive 20 mL portions of distilled water under vacuum.
    ___3. Filters are dried in oven at 103 to 105°C for at least 1 hour.
    ___4. After drying, filter (and gooches or weighing dishes) are stored in desiccator until cool.
    ___5. Balance is zeroed before weighing filters.
    ___6. Filters (and gooches or weighing dishes) are weighed before use.
___B. Sample Treatment
    ___1. Samples are well mixed and unrepresentative particles avoided when measuring volumes.
    ___2. Samples are filtered under vacuum.
    ___3. Sample volumes used yield no more than 200 mg total suspended solids.
    ___4. The solids capture is 1 mg, if 4.7 cm filters are used.
    ___5. After samples are filtered, filters are washed with 3 successive 10 mL portions of distilled water, dried (see #8 below), and cooled before weighing.
    ___6. Balance is zeroed before weighing residue
    ___7. Gooches or weighing dishes are handled with tongs.
    ___8. Samples are filtered so that plugging of filter is prevented.
    ___9. Samples are dried overnight, or drying cycle is repeated:
        ___Until constant weight is attained, or
        ___Until weight loss is than 4% of previous or 0.5 mg, whichever is less, or
        ___On occasion to check drying efficiency. Frequency:_____
    ___10. Drying time is determined after the oven is up to temperature.

TOTAL PHOSPHORUS, ASCORBIC ACID
EPA METHOD 365.2 EVALUATION CHECKLIST
(Method 4500- P B (5) and 4500-P E, Standard Methods, 18th ed.)

EQUIPMENT

___A. Spectrophotometer suitable for measurements at 880 or 650 nm, with a light path of at least 2.5 cm.
___B. Filter photometer equipped with a red color filter and a light path of 0.5 cm or longer.
___C. Hotplate or autoclave for digesting samples.

REAGENTS

___A. Digestion Reagents:
- ___1. 11 N sulfuric acid, H_2SO_4 for digesting samples made by diluting 300 mL of conc. H_2SO_4 with 1 L of distilled water.
- ___2. Ammonium persulfate, $(NH_4)_2S_2O_8$, or potassium $K_2S_2O_8$.
- ___3. 1 N sodium hydroxide, NaOH.
- ___4. Phenolphthalein indicator aqueous solution.

___B. Method Reagents:
- ___1. Potassium antimonyl tartrate solution:
  - ___Purchased commercially.
  - ___ Made in laboratory by dissolving 1.3715 g K $(SbO)C_4H_4O_6 \bullet {}^1/_2\ H_2O$ in 400 mL distilled water and diluting to 500 mL.
- ___2. Ammonium molybdate solution:
  - ___Purchased commercially.
  - ___ Made in laboratory by dissolving 20 g $(NH_4)_6Mo_7)O_{24} \bullet 1\ H_2O$ 500 mL distilled water.
- ___3. Ascorbic acid, 0.1 M:
  - ___Purchased commercially.
  - ___Prepared weekly in lab by dissolving 1.76g ascorbic acid in 100 mL distilled water.
  - ___Stored at 4°C.
- ___4. Combined Reagent:
  - ___The above reagents mixed in the following order and proportions for 100 mL of the combined reagent: 50 mL 5 N H_2SO_4, 5 mL of potassium antimoyl tartrate solution, 15 mL of ammonium molybdate solution, and 30 mL of ascorbic acid solution.
  - ___The reagents are at room temperature before they are mixed.
  - ___The solution is mixed after the addition of each reagent.
- ___5. Stock phosphate solution (50 mg/P/L, 1.0 mL=0.05 mg P)
  - ___Purchased commercially.
  - ___Prepared in lab dissolving 219.5 mg anhydrous KH_2PO_4 in dist. Water and diluting to 1 L.
  - ___Stored at 4°C.
  - ___Replaced as necessary
- ___6. Standard phosphate solution (0.5 mg P/L, 1.0 mL=0.5 µg P)
  - ___Purchased commercially.
  - ___Prepared in lab by diluting 10.0 mL of stock phosphate solution to 1000 mL with dist. water.
  - ___Other concentration and/or preparation: (specify)_ _
  - ___Stored at 4°C.
  - ___Replaced as necessary
- ___7. Working phosphate standards
  - ___Prepared in laboratory by mixed serial dilutions of the standard phosphate solution.
  - ___Cover at least the concentration range of 0.01 to 0.50 mg P/L for low level samples and 0.1 to 1.0 mg P/L for wastewater samples.

PROCEDURE

___A. Digestion
- ___1. 1 mL of 11 N H_2SO_4 and 0.4 g ammonium or potassium persulfate are added to the appropriate volume of samples, standards, and blanks.
- ___2. Treated samples, standards, and blanks are boiled on a hot plate for 30-40 minutes or autoclaved for 30 minutes at 15-20 psi. Samples are not boiled dry.
- ___3. Samples, standards, and blanks are then cooled, diluted, diluted, adjusted to pH 7.0$\pm$0.2 and diluted to

100 mL.

___B. Analysis

___1. 8.0 mL of combined reagent is mixed with 50.0 mL of samples, standards, and blanks.

___2. Color development is at least 10 min., but less than 30 min. before measuring absorbance or % T.

___3. Absorbance is measured at 880 or 650 nm.

___4. A reagent blank is used as the reference solution to set 0 Abs. or 100% T.

___5. A standard curve is prepared buy plotting the absorbance values of at least 3 different standards and a reagent blank vs. concentration.

___6. The standard curve is linear (correlation coeff. >0.995), and passes through the origin.

___7. The standards bracket the concentration range of the samples.

___8. A blank and a standard are analyzed with each series of samples.

___9. Calculations are done correctly.

__10. Results are reported as Total Phosphorus in mg/L.